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CYTOLOGICAL STUDIES IN THE MUSAE. I. MEIOSIS IN SOME TRIPLOID CLONES

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Received October 20, 1945

INTRODUCTION

SINCE 1932, an extensive program of banana breeding has been carried on by the Division of Botany of the JAMAICA DEPARTMENT OF AGRICULTURE with the primary aim of producing a variety of commercial value which is immune, or at least highly resistant, to Panama disease and leaf spot. While the main purpose of the program has not yet been satisfactorily achieved, a large body of information has been assembled which for the first time points the way toward reorganization of the program on a firmer scientific basis than has hitherto been possible owing to the lack of fundamental knowledge of the cytology, genetics, physiology and taxonomy of the Musae.

From the cytological point of view, the most important points which have come out of this work, together with that in Trinidad, are as follows: (1) The basic chromosome number of the Eumusae is n = 11 (CHEESMAN 1932; CHEESMAN and LARTER 1935, *contra* WHITE 1928). (2) The seeded bananas are diploid (2n = 22) as are some of the non-seeding edible varieties—for example, the apple and honey bananas of Jamaica. (3) The cultivated bananas and plantains of commerce are triploids (2n = 33). (4) Gros Michel (2n = 33) when crossed with a number of diploids gives a majority of tetraploid seedlings together with occasional heptaploids and diploids but very rarely viable aneuploids (LARTER 1935; CHEESMAN and DODDS 1942). (5) The predominant production of tetraploids does not hold for all triploid clones—for example, the red banana has given nothing higher than triploids, and Bluggoe (White House plantain) has so far shown a tendency to produce diploid offspring (CHEESMAN and DODDS 1942). (6) Both the Chinese (Canary) and Lacatan bananas have proved completely sterile (CHEESMAN 1932; LARTER (unpublished); CHEESMAN and DODDS 1942).

In view of these findings, the potential importance of detailed cytological studies in the Musae appears obvious. As a first step in this direction the writer has undertaken a general survey of meiosis in those clones which are at present of greatest practical interest. This survey has for convenience been divided into three phases: (1) A survey of meiosis in the naturally occurring diploids with special reference to those which have been used as male parents in the Jamaica breeding program. (2) A survey of several of the more important triploid clones with special reference to Gros Michel as the most obvious female parent and the apparently completely sterile Canary and Lacatan bananas. (3) A comparative survey of some of the more extensive seedling families.

Although all three phases of the survey have been carried on simultaneously, it has been found more satisfactory to deal with them separately. The

present paper, therefore, is concerned almost exclusively with the triploids for which suitable material was first available.

The following triploids have been used: (1) The Canary (China) and Lacatan bananas as representing the Cavendish-Robusta group. As noted, both these varieties appear to be completely sterile. (2) Gros Michel as representing the basic variety on which the banana growing industry of Central America and the Caribbean area is based. (3) The White House (Bluggoe) and Maiden plantains as representing that type of banana sometimes known as *Musa paradisica* which is normally eaten cooked.

The taxonomic relationships of these five varieties are in considerable doubt, and their separation into three groups is based more on general behavior and usage than on proper botanical classification.

The primary purpose of the present survey has been to lay a definite cytological foundation for further detailed studies rather than to attack any specific cytological problem. To that end some attempt has been made to assess the inter- and intra-varietal variations on a quantitative basis and where possible to correlate the findings with the known breeding behavior. Most of the results fall under one of the following heads: (1) First metaphase association—that is, organization and pairing relationships of the chromosomes on the first metaphase plate. (2) Chromosome segregation which covers the distribution of chromosomes from first anaphase to second telophase. (3) Pollen grain size especially in relation to the diploid standard Zebrina clone "A" (one of the *M. acuminata* Colla series). Statistical handling of the results has been carried out wherever such treatment seemed justified and informative. Otherwise raw data have been given. Many of the more interesting and in some cases more important points, however, cannot be expressed in quantitative terms and will, therefore, be discussed in the text and illustrated where possible with camera lucida drawings.

MATERIALS AND METHODS

The Canary banana and White House plantain were obtained from the botanist's variety collection at Hope, and each quite definitely represents a single clone. The first lot of Lacatan material was collected from a manurial trial at Grove Place Experiment Station, while the second lot came from Orange River Experiment Station. Gros Michel was collected from Caymanas Estate (Saint Catherine) and Potosi Estate (Saint Thomas). The first lot of Maiden plantain came from the Lands Department Centre, Grove Farm in Saint Catherine, and the second from Hampstead, Saint Mary. Where the materials have different origins, the results have for the most part been kept separate, since in several cases, especially with regard to the first metaphase picture, there appears to be slight differences between materials from different sections.

The technique employed was as follows: Whole flowers were fixed in three parts absolute alcohol to one part glacial acetic acid for 18 to 36 hours. Anthers were crushed in strong iron-acetocarmine, pressed, and mounted in a venetian turpentine mounting medium (Zirkle 1940; Wilson, in press). Pollen grain

measurements were made from temporary unpressed mounts with a micrometer eyepiece. A number of tests were carried out to determine the effect of fixation time on pollen grain size, and it was found that fixations varying from six hours to five days showed no significant differences.

Temporary unpressed mounts of first and second division stages were also examined in each of the varieties in order to check general cell characteristics and especially to determine whether cases of finding univalents well off the plate were natural or owing to pressure.

Most examinations were carried out with Zeiss 10× and 20× oculars and 90× 1.3 mm objective.

A $\frac{1}{12}$" Baker objective was substituted recently for the Zeis 90×, owing to breakage of the latter. Camera lucida drawings were made at various magnifications depending on illustration requirements.

OBSERVATIONS

General

The pollen mother cells of different varieties of Musa have several rather unique features in common, regardless of the degree of polyploidy. For instance, the nuclear volume is very small compared to the total cell volume (of the order of 1:20). Further, the most characteristic feature of the prophase nucleus is the presence of a single large deeply staining nucleolus. In the triploids this nucleolus appears to be intimately associated with about six chromosomes. In addition, the earlier stages of prophase (leptotene-pachytene) show an indeterminate number of smaller and fainter nucleolus-like bodies. From nine to 15 of these have been counted, but it is not impossible that there is one to each chromosome. In some cases, especially the Lacatan, there is another nucleolus-like body which is only slightly smaller than the "major" nucleolus. A third feature of the Musa chromosomes generally is the high degree of heteropycnosis at diplotene, especially in the Canary (see fig. 23), as well as at mitotic prophase in all varieties. Each chromosome appears to have two such regions proximally situated in each arm. A fourth feature of interest is the plane of the second division spindle which appears to be quite random, resulting in tetrads of all possible shapes.

First metaphase association

As many first metaphase plates as possible were examined in each variety to determine whether or not significant differences in the degree of pairing existed at this stage. In all cases, the results are highly variable, but there is little doubt that the five varieties fall into three distinct groups, especially as regards univalent-trivalent ratios (see tables 1 to 3). It has been difficult to find a statistical comparison which could be considered fair. Several methods have been tried, including comparison of means by the "t" test. This was checked by a modified goodness of fit (χ^2) test in which, for example, the distribution of trivalents in Gros Michel (table 1) was taken as a standard and the other varieties compared with it. The procedure was as follows: The

arrays were placed in four groups (0–2, 3–5, etc., trivalents) and the distribution compared with the expected on the Gros Michel standard. A similar analysis was made of univalents. Both forms of analysis showed a clear cut division of the five varieties into the three following groups:

(1) Low trivalent-high univalent formation. The Canary banana falls alone into this group. More than half the cells (45 out of 75) have no trivalents. The highest number of trivalents observed was five in two cases and the

TABLE 1

Distribution of trivalents in triploid musa.

VARIETY		NO. CELLS	0	1	2	3	4	5	6	7	8	9	10	11	
Canary		75	45	13	15	0	0	2	0	0	0	0	0	0	
Lacatan	Grove Place	26	1	1	21	3	—	—	—	—	—	—	—	—	
	Orange River	69	6	11	9	7	12	9	3	7	4	1	—	—	
	Total	95	7	12	30	10	12	9	3	7	4	1	—	—	
Gros Michel	Caymanas	43	—	4	30	9	—	—	—	—	—	—	—	—	8 cells disorganized
	Potosi	27	4	5	2	2	3	3	3	4	1	—	—	—	3 cells disorganized
	Total	70	4	9	32	11	3	3	3	4	1	—	—	—	
White House plantain		60	9	10	14	14	7	2	2	2	—	—	—	—	
Maiden plantain	Grove Farm	26	0	0	2	1	6	6	2	2	6	0	1	—	
	Hampstead	17	0	0	0	0	1	1	2	4	5	2	1	1	
	Total	43	0	0	2	1	7	7	4	6	11	2	2	1	

second highest two in 15 cases. The number of univalents is spread quite uniformly from nine to 33, with no class showing any very marked preponderance.

(2) Medium trivalent-univalent group. The Lacatan and Gros Michel bananas and the White House plantain fall into this group. They all tend to have from two to three trivalents in about half the cases, but the Lacatan has shown as many as nine, Gros Michel eight, and White House seven, and all have some in the zero class. Univalent distribution is similar in all three, with a tendency to peaks in the nine to 11 classes.

(3) High trivalent-low univalent group. The remaining variety, the Maiden plantain, falls into this group, with a trivalent distribution of two to 11 with the eight class having a slight majority. The univalent distribution covers

TABLE 2

Distribution of univalents in triploid musa.

VARIETY		NO. CELLS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Canary		75	—	—	—	—	—	—	2	—	—	7	3	4	2	4	2	1	2	2	—	7	2	5	1	5	1	4	—	8	—	4	—	3	—	6
Lacatan	Grove Place	26	—	—	—	—	—	—	—	—	2	11	1	7	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
	Orange River	69	—	—	1	4	6	3	9	12	5	7	6	3	2	1	—	—	1	2	—	—	2	1	1	1	—	—	—	—	—	1	—	—	—	1
	Total	95			1	4	6	3	9	12	7	18	7	10	3	4	—	—	1	2	—	—	2	1	1	1	—	—	—	—	—	1	—	—	—	2
Gros Michel	Caymanas	43				—	—	—	—	—	7	10	1	10	1	6	2	4	1	—	—	—	—	—	1			—							—
	Potosi	27				1	4	3	3	3	2	2	2	—	—	—	1							1	1			1							3
	Total	70				1	4	3	3	3	9	12	3	10	1	6	3	4	1					1	2			1							3
White House plantain		60					1	2	1	5	7	8	13	9	6	4	—	3	—	1															
Maiden plantain	Grove Farm	26	—	1	—	5	2	2	6	7	1	2																								
	Hampstead	17	1	1	2	5	4	2	1	1	—	—																								
	Total	43	1	2	2	10	6	4	7	8	1	2																								

classes zero to nine, with most cells falling into the four to seven classes.

These three groups are significantly different from each other, and it is of particular interest to note that the differences cut across the assumed taxonomic relationships.

Chromosome segregation

There are no marked quantitative differences between the five varieties with respect to the behavior of the chromosomes from first anaphase to second

TABLE 3

Distribution of bivalents in triploid musa.

VARIETY		NO. CELLS	0	1	2	3	4	5	6	7	8	9	10	11
Canary		75	5	3	5	10	7	6	7	9	5	9	5	3
Lacatan	Grove Place	26	1	—	—	—	—	—	1	3	9	11	1	—
	Orange River	69	1	1	3	4	7	8	11	13	5	8	6	2
	Total	95	2	1	3	4	7	8	12	16	14	19	7	2
Gros Michel	Caymanas	43	—	—	—	—	2	1	4	7	18	11	—	—
	Potosi	27	3	—	1	1	5	3	4	3	3	2	2	—
	Total	70	3	—	1	1	7	4	8	10	21	13	2	—
White House plantain		60	—	—	1	—	1	3	7	12	12	11	10	3
Maiden plantain	Grove Farm	26	—	1	1	5	3	1	6	6	1	2	—	—
	Hampstead	17	1	1	2	5	4	2	1	1	—		—	—
	Total	43	1	2	3	10	7	3	7	7	1	2	—	—

telophase. As would be expected, most first anaphases show a number of lagging chromosomes, and not infrequently one or more lie well away from the spindle area entirely. As far as the main chromosome groups are concerned, second division is quite normal in most cases—that is, a normal second metaphase plate is formed and second anaphase segregation occurs without misdivision. The only exceptions to this are occasional second anaphases in Gros Michel and more especially in Lacatan which show unequal divisions and/or one or two laggards.

The behavior of "omitted" chromosomes is in itself interesting. Most of them survive either in small groups or even singly, depending on their relative positions, as micro-nuclei which separate as micro-cells. Although several

hundred second telophases and tetrads have been examined carefully, only one case was found in which a cell was binucleate.

Tetrad formation

The typical "tetrad" in the triploids consists of four large cells and a varying number of small ones. Some of the small ones are so minute as to make it very difficult to do an accurate count of distribution of number of cells per tetrad. The distribution figures given in table 6, however, were derived from the clearest material and probably represent existing conditions fairly accurately. In any case they are of undoubted comparative value. The Canary banana and the two plantains have definite peaks at the five-cell class, the Lacatan tends to have about equal numbers in the five- and six-cell classes, and Gros Michel in the six- and seven-cell classes. The present figures also indi cate that the White House plantain may have a second peak in the seven class, but further investigation is necessary before this can be considered to be definite. In general, both Lacatan and Gros Michel tend to have more cells per tetrad than the other varieties. As will be seen later, this condition is not unexpected.

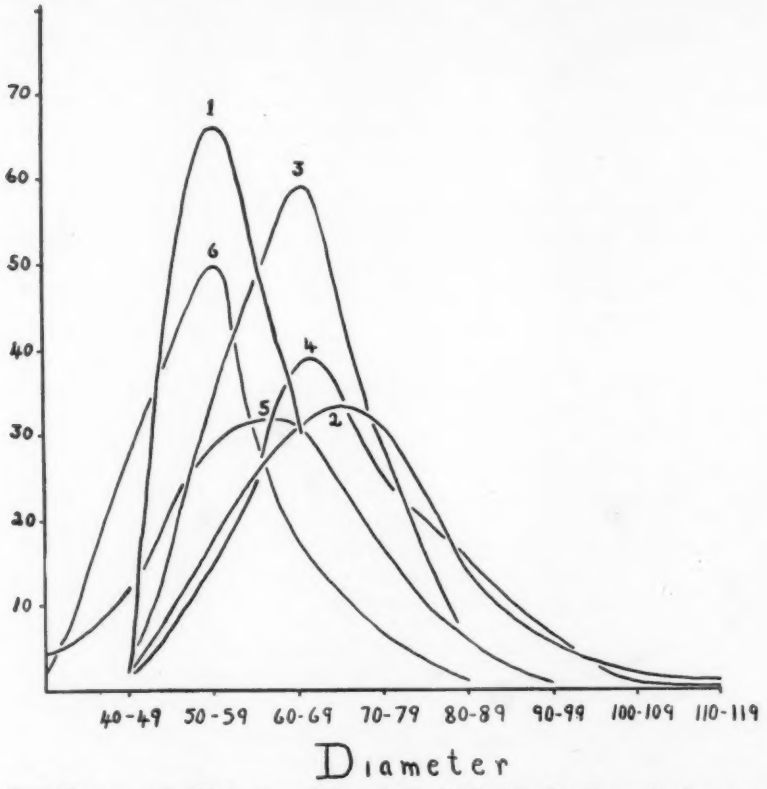
Pollen grain size

The diameters of morphologically normal pollen were measured in each variety chiefly to determine whether or not the size distribution is reasonably normal (table 1 and text fig. 1). As may be seen from text figure 1, there is no marked departure from a normal distribution in any of the varieties. Perhaps the most interesting point is the similarity between the Maiden plantain and the diploid "Zebrina," the main difference between them being the greater range of sizes in the former. As far as mean sizes are concerned, there are three groups (table 7): large (Canary and Gros Michel); medium (Lacatan and White House); and small (Maiden plantain).

Some significant aberrations

The above results give the impression that the three varieties Lacatan, Gros Michel and White House plantain are virtually indistinguishable cytologically. This is not quite so. Gros Michel, for instance, shows a type of aberration which is present only very rarely in Lacatan and, as far as is known, not at all in the White House plantain. Some eleven cells have been found in Gros Michel at first meta-anaphase in which the chromosomes show a type of disorganization which may be of considerable significance in relation to its breeding behavior. In these cases the chromosomes are not normally aligned on the plate but point in all directions and exhibit a considerable degree of desynapsis (*cf.* HUSKINS and WILSON 1938). Further, the spindle is always ill defined and in most cases appears to be lacking altogether. In some cases premature separations have proceeded to the point where "sister" chromatids are separated. The suggestion is that such cells are the precursors of the restitution nuclei postulated to explain the formation of triploid gametes which is relatively common in this variety. A similar breakdown occasionally occurs at second meta-

phase. Probably the most significant observation in this connection is a second metaphase (fig. 22) containing 30 chromosomes on the plate with the other three scattered about. All 33 chromosomes were within a single cell wall. Such a cell if second metaphase is normal will certainly give a 30-chromosome gamete and possibly a 33-chromosome one. A similar but less marked tendency



TEXT FIGURE 1. Graphical representation of the distribution of pollen grain characters. 1. Zebrina "A," 2. Canary, 3. Lacatan, 4. Gros Michel, 5. White House, 6. Maiden plantain.

was discovered in Lacatan. The Canary banana also showed complete asynapsis in several cases but without the marked spindle disorganization. Neither of the plantains showed any such tendency.

The behavior of "omitted" or "lagging" chromosomes (they are not always univalents) is of some interest. In all cases they are variable in behavior. Some split at first anaphase (see especially fig. 21), others do not seem to split at all, and some fall apart subsequent to first anaphase of the main group. In Gros Michel fully half the "omitted" chromosomes go through a definite sec-

ond division with a properly formed spindle. Even single chromosomes have been seen to go through a perfectly normal division. A similar condition has been observed in Lacatan but more rarely. This does not seem to be characteristic of the other varieties. This difference between Lacatan and Gros

FIGURES 1–5.—First metaphase plates of Canary, Lacatan, Gros Michel, White House, and Maiden plantains, respectively. Fig. 1, 2, 3 and 5 ca. 1200×, fig. 4 ca 1600×.

Michel and the other varieties seems a satisfactory explanation of the greater number of cells per tetrad which the former varieties tend to have.

DISCUSSION

During the present investigation two rather general questions have been kept in mind: (1) How extensive and of what kind are the variations within and between varieties, and (2) what is the extent of the correlation between the meiotic behavior and the breeding characteristics? It is impossible with the data so far obtained to answer either question fully, but certain relevant points seem well worth stressing.

In relation to the first question there is clear and unequivocal evidence that the five triploid varieties fall into three groups especially with regard to their pairing relations at first metaphase. The Canary banana shows reduced pairing of all three chromosome sets. Only 20 percent (table 4) of the cells give 11 associations most of which are bivalents. The suggestion from this data is that the Canary banana has two semi-homologous chromosome

sets plus a third set (ignoring the two cases of five trivalents) with only two chromosomes having strict homologues. In short, this variety might well be symbolized as an approach to an *A A B* type. The Lacatan and Gros Michel bananas and the White House plantain appear to have two quite homologous sets plus a third semi-homologous set which might be symbolized as *A A* A_1.

FIGURES 6–10.—First anaphases of Canary, Lacatan, Gros Michel, White House and Maiden plantains.

Note lagging trivalent in fig. 6, lagging bivalent in fig. 7, and splitting univalents in fig. 8 and 9. Fig. 6 ca 1600×, fig. 7 ca 600×, fig. 8–10 ca 1200×.

The Maiden plantain in contrast tends strongly to the autotriploid and may be characterized as an approach to an *A A A* type. Questions as to the cause or origin of these differences require very extensive and complex cytotaxonomic and cytogenetical investigations beyond the scope of the presently available facilities. In view of the present breeding aim, the question of the evolutionary origin and development of the triploids is one of some importance, but the problem is a complex one, if for no other reason than the fact that the banana

TABLE 4

Total number of first metaphase plates with 11 associations.

VARIETY		NO. CELLS	ELEVEN ASSOCIATIONS	PERCENTAGE
Canary		75	15	20.0%
Lacatan	Grove Place	26	14	53.8%
	Orange River	69	26	37.8%
	Total	95	40	42.1%
Gros Michel	Caymanas	43	17	39.5%
	Potosi	27	20	74.0%
	Total	70	37	52.8%
White House plantain		60	29	47.3%
Maiden plantain	Grove Farm	26	24	92.4%
	Hampstead	17	17	100.0%
	Total	43	14	95.3%

has been cultivated virtually since prehistoric times. There is no real basis for even guarded speculations on the question of whether the various edible triploids have a common origin and have separated by mutation processes or were separately evolved. Two possible modes of origin are (1) the production of a tetraploid by spontaneous chromosome doubling in the zygote or seed and subsequent crossing with a like or unlike diploid, and (2) the formation of a diploid gamete by suppression of the first division of meiosis (as postulated for Gros Michel) and subsequent union with a haploid gamete. Failure to find any wild tetraploids plus the behavior of Gros Michel tends to favor the second possibility.

Until the taxonomic muddle into which the Musae have fallen is resolved and large scale cytogenetical investigations have been carried out, further

TABLE 5

*Chromosome segregation.**

VARIETY		NO. POLES	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	"LOST" CHROMOSOMES	
																													MEAN	RANGE
Canary		45				1	—	1	1	5	4	9	8	6	3	4	2	1											4.6	0–10
Lacatan	Grove Place	46	—	—	—	—	1	—	—	5	5	18	7	6	—	2	1											1	4.6	0–10
	Orange River	126	4	—	1	1	1	1	3	8	11	23	26	15	19	5	4	4											4.2	0–23
	Total	172	4	—	1	1	1	1	3	13	16	41	33	21	19	7	5	4										1		
Gros Michel	Caymanas	95			1	3	2	2	3	16	18	19	15	6	—	3	3	—	2	1	—	—	1						5.3	0– 8
	Potosi	57			1	—	—	3	—	5	6	10	11	10	6	4											—	1	3.4	0–16
	Total	152			2	3	2	5	3	21	24	29	26	16	6	7	3	—	2	1	—	—	1	—	—	—	—	1		
White House plantain		98						2	7	9	14	9	17	18	10	8	2	2											3.4	0–11
Maiden plantain	Grove Farm	31							1	7	3	5	2	9	3	1													4.8	0– 8
	Hampstead	22							1	—	—	2	7	5	5	2													1.6	0– 5
	Total	53							2	7	3	7	9	14	8	3														

* Combined figures from AI, MII and AII.

speculation on origins and evolutionary development is quite profitless.

The relationship between the cytological behavior of the five varieties and their breeding behavior remains obscure to a certain degree. Nothing, so far

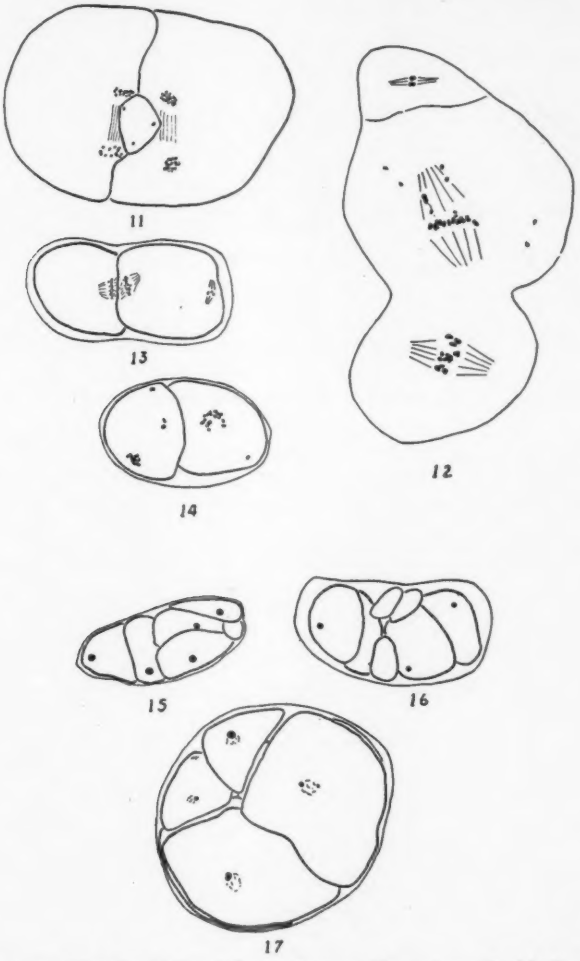


FIGURE 11.—Second anaphase of Canary banana. Note micro-cell with three scattered chromosomes.

FIGURE 12.—Second metaphase of Lacatan banana. Note supernumerary spindle at top.

FIGURE 13.—Second metaphase of Gros Michel.

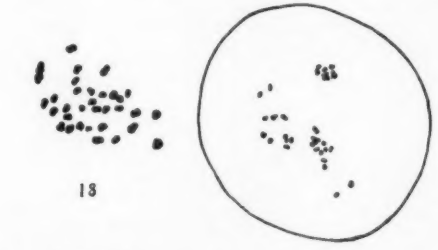
FIGURE 14.—First telophase of White House plantain.

FIGURES 15–17.—Tetrads from Canary, Gros Michel and Maiden plantain, respectively. Fig. 11, 13, 14, ca 450×, fig. 12 ca 540, fig. 15 ca 220, fig. 16, 17 ca 450×.

TABLE 6

Cells per "tetrad."

VARIETY		NO. TETS.	CELLS PER TETRAD										PEAK
			2	3	4	5	6	7	8	9	10	11	
Canary		250		2	60	98	39	40	5	6	—	—	5
Lacatan	Grove Place	100		—	11	20	52	16	1	—	—	—	
	Orange River	100		—	22	43	22	11	2	—	—	—	
	Total	200		—	33	63	74	27	3	—	—	—	5–6
Gros Michel	Caymanas	123		—	2	25	55	37	3	1	—	—	
	Potosi	127		—	6	10	29	44	22	12	3	1	
	Total	200		—	8	35	84	81	25	13	3	1	6–7
White House plantain		200		—	23	70	37	54	9	7	—	—	5–7
Maiden plantain	Grove Farm	100	2	2	13	29	27	20	6	0	1	—	
	Hampstead	50	—	—	12	24	9	4	1	0	0	—	
	Total	150	2	2	25	53	36	24	7	0	0	—	5



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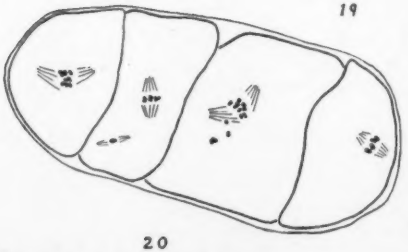

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FIGURE 18.—Asynaptic figure from Canary banana.

FIGURE 19.—Disorganized Gros Michel first meta-anaphase nucleus.

FIGURE 20.—Multi-spindle second metaphase from Gros Michel. Fig. 18 ca 1370×, fig. 19, 20 ca 510×.

as the writer is aware, is known concerning the breeding behavior of the Maiden plantain. The remaining four varieties fall into three categories: (1) the Canary and Lacatan bananas which are apparently completely sterile. (2) Gros Michel which occasionally seeds in crosses with diploids to produce

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FIGURE 21.—First anaphase from Lacatan. Note splitting univalents still on plate.

usually tetraploids, occasional heptaploids, a few diploids (only four have been detected in several thousand seedlings), and some viable aneuploids. (3) The White House plantain which seeds to a small extent with a variety of diploids to give diploid, triploid, tetraploid and, some aneuploid seedlings. CHEESMAN and DODDS (1942) find the diploids in the ascendency. The num-

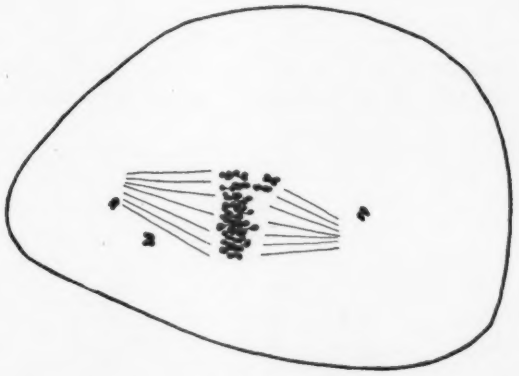

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FIGURE 22.—Mononucleate second metaphase from Gros Michel. Note plate contains 30 chromosomes with the other three scattered and retaining first anaphase appearance.

ber of germinations is rather low in all cases. Out of some 600 seeds only about 50 germinated in CHEESMAN and DODDS' work. Out of 50 seeds obtained in a similar cross here only two germinated and only one survived potting.

The cytological picture in both the Canary and Lacatan bananas is cer-

TABLE 7

Pollen grain size.

VARIETY		NO. CELLS	MEAN	S.E.
Canary		200	70.4	1.93
Lacatan	Grove Place	100	62.4	
	Orange River	150	64.9	
	Total	250	63.9	.51
Gros Michel	Caymanas	100	67.2	
	Potosi	100	72.6	
	Total	200	69.9	.82
White House plantain		200	60.5	.78
Maiden plantain	Grove Farm	100	55.0	.30
Zebrina "A"		200	57.0	.27

tainly not conducive to high fertility. For instance, the microspore of the Canary banana may roughly be considered as having chromosome contents derived from random segregation of 33 univalents. That with adjustment for laggards, makes the chances of obtaining a balanced genic complement of euploid number approach the order of 1:225,000. The Lacatan with 40 per-

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FIGURE 23.—Diplotene of Canary banana showing strong heteropycnosis. Fig. 21, 22 ca 1370×, fig. 23 ca 450 ×.

cent of the metaphases having 11 associations (table 4) has a better chance of producing microspores of complete genic complement, but these would only very rarely be euploid. The Canary banana shows no indication of special mechanisms such as Gros Michel's production of restitution nuclei. The Lacatan on the other hand has the Gros Michel tendency but to a much less marked degree.

The production of triploid and hexaploid gametes in Gros Michel is almost certainly the result of occasional complete suppression of either one or both divisions of meiosis. Aneuploid gametes approaching the triploid or hexaploid condition have a like explanation. The formation of haploid gametes, however, must be tentatively put down to chance operation on the normal chromosome segregation.

Functional haploid gametes in the White House plantain appear to be more frequent than in Gros Michel. Why this should be so is not clear, nor is it clear why this variety should be so much more fertile than the cytologically similar Lacatan. There does not appear to be any special mechanism operating (for example, a tripolar spindle) to produce such a result.

In general, the breeding behavior of Gros Michel is quite compatible with its cytology. The differential between Lacatan and White House has so far found no satisfactory cytological explanation, though it is not certain that some such explanation does not exist possibly in the summation of small differences which the present survey was not extensive enough to uncover. On the other hand, the explanation may be in genetic relationships of which nothing is known. Certainly the testing of Lacatan against a wide range of diploids is indicated as is the *in vivo* behavior of wild pollen on Lacatan stigmas.

SUMMARY

Meiosis of five triploid clones of Musa (Canary, Lacatan, and Gros Michel bananas and the White House and Maiden plantains) has been examined in order to assess inter- and intra-varietal variations and to determine the relationships between their cytological and breeding behaviors.

The cytological picture found in the Canary banana appears to be sufficient explanation for its apparent complete sterility. Certainly many thousands of pollinations must be made to have even a theoretical chance of obtaining one seed.

The cytological behavior of Gros Michel is completely in accord with its normal breeding behavior. Suppression of the first division of meiosis leads to triploid gametes which could combine with haploid gametes from the wild parent to give tetraploid offspring. Practical breeding gives this result in the vast majority of cases.

The Lacatan banana and the White House plantain are similar cytologically but differ markedly in their breeding behaviors. The former is quite as sterile as the Canary banana, while most pollinated stems of the latter yield some seed, the majority among the viable ones being diploid. Whether this difference can be attributed to the summation of slight ill-defined cytological

differences not easily detected in such a survey as the present one or whether the explanation is largely physiogenetical is not yet clear.

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ON THE DOT CHROMOSOMES OF DROSOPHILA REPLETA AND D. HYDEI

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INTRODUCTION

DROSOPHILA HYDEI Sturtevant and *D. repleta* Wollaston[1] are closely related species, both frequent in association with man in many regions—at least throughout the United States, where both species are evidently of relatively recent introduction. There are numerous other species of the same group, mostly found in the Neotropical region and in the southern portion of the United States. All these agree in having a pale gray pollinose dorsal surface of the mesonotum, each hair and bristle arising from a dark blackish-brown non-pollinose spot. This spotted pattern has furnished the basis for most of the studies reported here.

D. repleta has the chromosome configuration that is most characteristic for the subgenus Drosophila to which it belongs—five pairs of rods (one of which is X) and a pair of dot-like chromosomes. It is, of course, probable that each of these chromosomes also has a short heterochromatic arm (see STURTEVANT and NOVITSKI 1941). *D. hydei* differs in its metaphase configuration only in that there is an additional arm visible on the X; this arm appears to be wholly heterochromatic. *D. neorepleta* Patterson and Wheeler, from Guatemala, resembles *repleta* in its external characters even more than does *hydei;* it differs from *repleta* cytologically in that one of the rod-shaped autosomes has become J-shaped—presumably through the occurrence of a pericentric inversion. For our present purposes the significant point is that the dot chromosome, or element F (STURTEVANT and NOVITSKI 1941), is present in all three species (for *neorepleta* see footnote on p. 261).

D. REPLETA

DR. E. NOVITSKI subjected *D. repleta* to X-rays and obtained a dominant mutant gene, called "Stubble" (symbol *Sb*). The Stubble phenotype somewhat resembles the character bearing the same name in *melanogaster*, but is less extreme. The bristles are shortened and taper less than do those of wild type. There is never any difficulty in classifying for *Sb*. *Sb* is lethal when

[1] This name is well established in the literature, but may be incorrect. The type specimens, from Madeira, I saw at the British Museum in 1922. At that time I was aware of the existence of only four species in this group, and these specimens most resembled the species now given this name; they were, however, surprisingly small and dark. I am now inclined to suspect that they represent some other species—perhaps *D. buzzatii* Patterson and Wheeler. However, if this be so, the correct name to apply here will still be in doubt, since there are several supposed synonyms that will be difficult to identify with certainty. For the present it seems best to retain the name *repleta*.

homozygous, and was long kept in a selected stock, but has now been balanced by a recessive lethal that arose in a *Sb*/+ stock.

On outcrossing *Sb* to wild type, I have found that a new phenotype is regularly produced. This type, which may be called "Diminished" (*Dm*), is characterized as follows: darker than wild type, owing to an extension of the dark spots surrounding the bases of the hairs and bristles; smaller than wild type; bristles rather blunt, but by no means Stubble; furrow between mesonotum and scutellum deep; both sexes wholly sterile. Table 1 shows the frequencies with which this type was produced.

TABLE 1

Offspring of Stubble flies.

MATING	*Sb*	+	*Dm*	*Dm Sb*	TOTAL
+ ♀ × *Sb* ♂	485	446	55	0	986
Sb ♀ × + ♂	197	207	49	1	454
Sb ♀ × *Sb* ♂	338	120	20	15	493

Taking into account the X-ray origin of Stubble, these results leave little doubt that we are dealing with a translocation. The Diminished flies then are aneuploid, and there is presumably a complementary class that is inviable. Since males and females are approximately equally frequent among both *Sb* and *Dm* flies in all three crosses, the two chromosomes involved are evidently both autosomes. The most likely situation then is that one of them is the dot, most of the genes of which have been exchanged for a small portion of one of the four rod-shaped autosomes. The *Dm* flies then are haploid for most of the dot, and triploid for the small portion of a rod that is now attached to the dot centromere. This interpretation also accounts for the *Dm Sb* flies; they carry a normal rod, both translocated chromosomes (two of the shorter one, one of the longer one; the latter is responsible for the *Sb* phenotype), but no normal dot. In the second cross the individual of this type was due to primary non-disjunction of the dot in the wild type father; presumably it had only one small translocated chromosome. This interpretation is confirmed by the observation that sterile individuals having this phenotype are occasionally found in pure wild type stocks, both of *repleta* and of *neorepleta*, and among the hybrids discussed below.

The frequency with which the "irregular" type of segregation occurs in *Sb* flies can hardly be determined, since the *Dm* flies seem to be distinctly less viable than their + or *Sb* sibs; it may be noted, however, that the segregation seems to be more often regular in the males (5.6 percent *Dm*) than in the females (10.8 per cent *Dm*).

It was first shown by NOVITSKI (unpublished data) that the mating *Drosophila neorepleta* ♀ × *D. repleta* ♂ occasionally produces a few offspring. Neither NOVITSKI nor I have ever been able to get any offspring from the reciprocal mating. WHARTON (1942, 1944) reports the same result, though the table in the latter paper (p. 177) gives the reverse result. Miss WHARTON in-

forms me (private communication) that her observations agree with ours and with her text, not with her table.

The F_1 hybrids are rarely produced, and the males are all wholly sterile, with poorly developed testes; but the females occasionally produce a few offspring when mated to *repleta* males—never, in my experience, when mated to *neorepleta* males. Some of the females from this backcross are moderately fertile, and a study of their genetic behavior will be reported elsewhere. For present purposes the significant point is that hybrid strains from such a source have repeatedly produced a light-colored type that behaves as though it were due to a dominant gene, and have also occasionally produced a sterile dark type that closely resembles the one derived from Stubble.

The Light (symbol *Lt*) specimens may be supposed to have three dot chromosomes, since their pattern differs from that of wild type in the opposite direction from that of *Dm*. Presumably when first produced they carry two dots derived from *repleta* and one from *neorepleta*.[2] After repeated backcrossing to *repleta* it is more likely that all three are of *repleta* origin; no phenotypic difference has been observed that could be attributed to such a difference in origin of the dots.

When *Lt*×+ or +×*Lt* matings are made, there result *Lt* and+flies in approximately equal numbers in both sexes. The mating of *Lt*×*Lt* gave 576 *Lt*: 229+; but it was observed that some of the Light flies from such matings are more extreme than the usual type. The dark spots have almost disappeared, and the groove between the mesonotum and scutellum is very shallow —again a character opposite in sign to that found in *Dm*. Such extreme Light flies are sterile. They evidently carry four dot chromosomes. In the crosses where their identification was attempted, the count was: *Lt*♀×*Lt*♂→81 Extreme *Lt*, 337 *Lt*, 161+. Evidently the viability of tetra-dot is about one-half that of triplo-dot or of wild type.

Lt and *Sb* were crossed, and it was observed that flies showing both characters had the Stubble phenotype fully developed. Unfortunately the genetic tests of such *Lt Sb* flies were not carried out extensively, nor were the matings planned to give the simplest analysis; accordingly it does not seem necessary to present what data were obtained.

In the absence of these critical crosses, and in the absence of all cytological data, the evidence for the interpretation of the *Dm* and *Lt* types (as being haplo-dot and triplo-dot, respectively) is not conclusive. There can, I think, nevertheless be no doubt that such is their nature, since their properties are in such good agreement with the data from *hydei*, now to be described.

D. HYDEI

D. hydei is closely related to *D. repleta* and resembles it in all essential respects that concern characters used in identifying the *Dm* and *Lt* types of

[2] WHARTON (1942) shows that there is no dot-shaped chromosome in *neorepleta;* her observations are confirmed by DR. NOVITSKI. It is evident, however, from the chromosome numbers and from WHARTON'S report on the salivary gland chromosomes, that element F is present as a separate chromosome, but has acquired enough heterochromatin to make it impossible to identify it in metaphase figures.

repleta; it was to be expected, therefore, that the corresponding types of *hydei* could be worked with. This expectation has been partially fulfilled for *Lt*, but *Dm* has not been observed. The chief reason for turning to *hydei* is the existence of strains carrying mutant genes located in the dot chromosome. The two types, both discovered and located in the dot by DR. W. P. SPENCER, but not yet described, were supplied by him, along with his generous permission to use them and to publish his localization of them.

One of these types, Extended (symbol *Ex*), is characterized by an extension of the dark spots at the bases of the hairs and bristles. In homozygous Extended the gray ground of the mesonotum and scutellum is wholly obliterated, producing a blackish-brown fly, and the head and thorax are also somewhat abnormal in shape. In *Ex*/+ the shape is normal, but there is a marked and easily identified darkening of the mesonotum, especially along its lateral margins. A sample count from *Ex*/+ × *Ex*/+ gave 51 *Ex*/*Ex*: 100 *Ex*/+ : 45 + —indicating approximately equal viability for all three classes.

The other mutant strain received from SPENCER apparently carries two mutant genes in the same dot chromosome—though the two have not been separated by crossing over. One is a recessive, grooveless, very similar to the dot chromosome character of the same name in *melanogaster* and *pseudoobscura*. This character is rarely seen, since most flies homozygous for the chromosome in question fail to survive; no use was made of the grooveless character in the present studies. The other mutant gene in this chromosome is a dominant called Cubitus interruptus (*Ci*). In *Ci*/+ flies, the fifth vein is interrupted between the posterior crossvein and the anal crossvein—this section is in fact usually less than half present. In *Ci*/*Ci* flies, when they occasionally survive, the character is similar, but distinctly more extreme, and includes a slight effect on the fourth vein. The parallelism to the cubitus interruptus of *melanogaster* may be questioned, since that character involves the fourth vein much more markedly than it does the fifth.[3] *Ci*, however, does closely resemble the description and figure given by CHINO and KIKKAWA (1933) for the dominant Gap in the dot chromosome of *virilis*.

From two cultures of *Ci*/*Ex* mated together the counts were: 214 *Ci*/*Ex*, 86 *Ex*/*Ex*. The *Ci* chromosome here, as usual, acted as a lethal, and the expected ratio therefore is 2:1. There is a slight deficiency of *Ex*/*Ex*, which was shown above to have (in another experiment) normal viability. It may be concluded that heterozygosis for *Ci* does not decrease the viability. It should be added that I have never detected crossing over between *Ci* and *Ex*, though no extensive tests have been made.

The first tests for non-disjunction of the dot were carried out with *Ex*. Since *Ex*/+ resembles the haplo-dot of *repleta*, the most hopeful test seemed to be that based on the assumption that +/+/*Ex* would be more nearly wild type in pattern than is +/*Ex*—an expectation that was in fact realized. Wild type

[3] This discrepancy raises a question as to the appropriateness of the name applied. It would appear, however, that the fifth vein, rather than the fourth, is best considered the cubital. The type with the questionable name then is the *melanogaster* one rather than the *hydei* one; and the name is now too well established in the genetic literature on *melanogaster* to be changed.

strains were crossed, to produce +/+ flies in which the dot chromosomes were of diverse geographical origins, and such flies were then mated to *Ex/Ex*. All the regular offspring are expected to be +/*Ex*, but if non-disjunction occurs in the +/+ parent, +/+ gametes will give +/+/*Ex* flies. The results obtained are shown in table 2.

TABLE 2

Tests for primary non-disjunction of dot chromosomes in D. hydei.

SOURCE OF + CHROMOSOMES	SEX OF WILD TYPE PARENT	+/*Ex* OFFSPRING	+/+/*Ex* OFFSPRING
Alabama; Iowa	♀	755	0
Alabama; Iowa	♂	511	0
Yucatan; Iowa	♀	892	9

The nine +/+/*Ex* were all from a single pair mating (among six of this composition) and may perhaps have been due to an irregular somatic division, rather than to nine separate meiotic ones. They had 141 +/*Ex* sibs. Phenotypically these flies were intermediate between +/+ and +/*Ex*. Later experience has indicated that they can be separated from the latter with little difficulty, but not always with complete certainty from the former—though it is usually possible to be reasonably certain of the composition of a few individuals of each class. Accordingly, in table 3 three classes are recorded: "+," "+/*Ex*," "*Ex*." The two latter are both diplo-dot in the first four crosses; the first includes +/+/+, +/+, and +/+/*Ex*; and the "+/*Ex*" class from the last cross also includes +/*Ex*/*Ex*.

TABLE 3

Offspring of triplo-dot hydei carrying Extended.

♀ PARENT	♂ PARENT	"+"	"+/*Ex*"	"*Ex*"	TOTAL	% *Ex* GAMETES
+/+/*Ex*	+/+	167	28	0	195	14.4
+/+	+/+/*Ex*	193	91	0	284	32.1
+/+/*Ex*	*Ex*/*Ex*	105	924	164	1193	13.8
Ex/*Ex*	+/+/*Ex*	70	145	68	283	24.1
+/+/*Ex*	+/+/*Ex*	576	318	47	941	—

The +/*Ex*/*Ex* class is darker than +/*Ex*, but it has not been found possible to distinguish the two in every case—though again it is possible to select individuals that are clearly of either composition. The +/+/+ class corresponds to the Light type of *repleta* and agrees with it in having smaller dark spots. It is, however, less sharply different from wild type, and here also it has been found possible only to identify some of the more extreme individuals.

To recapitulate: the order of increasing darkness (or increasing size of

dark spots) is: +/+/+, +/+, +/+/*Ex*, +/*Ex*, +/*Ex*/*Ex*, *Ex*/*Ex*. The *Ex*/*Ex*/*Ex* class has not been identified. In this seriation only two of the intervals are sharp enough to allow certain separations—that between +/+/*Ex* and +/*Ex* and that between +/*Ex*/*Ex* and *Ex*/*Ex*.

Triplo-dot specimens from these experiments were crossed to the *Ci* strain, and it was found that *Ci*/+/+ is intermediate between +/+ and *Ci*/+; unfortunately it overlaps both of these classes, so that counts were found to be quite unreliable—though once again some individuals could be identified, as was shown by genetic tests involving *Ex*. The *Ci*/*Ci*/+ class was also produced; it showed the *Ci* character in extreme form, but could not be sharply separated from *Ci*/+. Because of these classification difficulties it does not seem desirable to record the crosses that were made. These experiments, however, were adequate to confirm the interpretation of the *Ex* experiments as being due to an extra dot chromosome, rather than to an independently inherited inhibitor of *Ex*.

The final proof of the correctness of the interpretation, however, was obtained by cytological methods. Dr. K. W. Cooper examined the neuroblast cells of three larvae from a mating of +/+/*Ex* ×+/+/*Ex* and found two of them to have three dot chromosomes (fig. 1a), while the third had four (fig. 1b). This tetra-dot specimen raises the problem of whether or not such individuals survive to the imaginal stage, as they evidently do in *repleta*. No specimens were so identified, and the data of table 3 suggest that they are not fully viable; the question whether or not they ever survive cannot yet be answered.

The data of table 3 indicate that, in *hydei* as in *melanogaster*, segregation is not random in triplo-dot flies. With random segregation the mating between +/+/*Ex* and *Ex*/*Ex* (in either direction) should give 2+/*Ex*/*Ex*: 2+/*Ex*: 1+/+/*Ex*: 1*Ex*/*Ex*—or, grouping the classes that cannot be distinguished, 1"+": 4"+/*Ex*": 1"*Ex*." Mating of +/+/*Ex* to +/+ should give, after similar grouping, 5"+": 1"+/*Ex*." Examination of the table shows that this result is approximated by the triplo-dot females, though there is probably a real deficiency of ++−*Ex* segregation as compared to +−+*Ex*. In the males, however, there is a distinctly more marked *excess* of ++−*Ex* segregation. Unfortunately the "+" chromosomes in the present experiments came from a variety of sources, with the result that a detailed analysis of the preferential segregation, similar to that which has been made for *melanogaster* (Sturtevant 1936), cannot be made until more carefully designed experiments are carried out. Such experiments were planned, but when triplo-dot *Ci* turned out to be unclassifiable it was evident that no satisfactory test of the segregation-frequency interrelations could be made, and the experiments were not carried out. It should be indicated, however, that the observed sex difference in preference values is opposite in sign to that found in *melanogaster*, where males, rather than females, give nearly random segregation in triplo-dot experiments. In *hydei* this difference has been consistently present and of the same sign in experiments where the "+" chromosomes differed in origin from one experiment to another, but were alike in the males and females compared.

COMPARISON OF D. REPLETA AND D. HYDEI

As indicated above, triplo-dot is more easily distinguished from wild type (diplo-dot) in *repleta* than in *hydei*. This statement, however, needs some qualification. Several wild stocks of *repleta* (from Pasadena; Austin, Tex.; St. Louis, Mo.; Detroit, Mich.; Newcastle, Pa.) have been found to be variable in the extent and intensity of the dark thoracic markings. Selection within these strains has easily established rather uniformly dark types that are perhaps

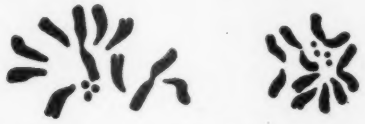

FIGURE 1.—Ganglion cells from two female larvae of *Drosophila hydei*. Triplo-dot (left) and tetra-dot (right). Preparations and drawings by Dr. K. W. COOPER.

slightly darker than the usual *neorepleta* or *hydei* types; the work here recorded has involved only such dark strains—which represent the most usual form of the species. Selection of the lighter types has resulted in strains lighter than the triplo-dot individuals here described. It is clear that numerous genes are involved here, but few if any of them appear to be in the dot chromosome. In any case, it is necessary to avoid such strains if clear-cut separations for triplo-dot flies are desired.

These extreme light selected strains bear a superficial resemblance to the sex-linked recessive described by STURTEVANT (1915) (see also MORGAN, BRIDGES, and STURTEVANT 1925), but that gene has not been found in any of the stocks used in these experiments. The extreme light types also bear a phenotypic resemblance to the form of *hydei* described by SPENCER (1940) as subspecies *yucatanensis*. It happens that one of the + chromosomes used in the *hydei* experiments was derived from *yucatanensis* (see table 2); this chromosome had been transferred to a California background by repeated crosses to an Extended stock, and the results show that little if any of the lightness of *yucatanensis* is due to its dot-chromosome. With the exception of the strains of *yucatanensis* (from Chichen-Itza), I have not found *hydei* to be particularly variable in its thoracic pattern—certainly the strains I have had are much less so than are most wild strains of *repleta*.

The greater ease of separation of triplo-dot in *repleta* is therefore present in spite of a somewhat greater variability of the wild type with which it is compared.

Two other differences between the two species are probable but less certain —namely, that both tetra-dot and haplo-dot are more viable in *repleta*. The former was identified in a *hydei* larva, but not in adults; the absence of the latter may be due only to lack of efficient methods of identifying it.

COMPARISON WITH SPECIES OF DROSOPHILA NOT IN THE REPLETA GROUP

Haplo-dot individuals have been identified and described in *melanogaster* (BRIDGES 1921), *simulans* (STURTEVANT 1929), *ananassae* (KIKKAWA 1938), and *virilis* (CHINO and KIKKAWA 1933; CHINO 1937).

In all these species the type is characterized by "minute" bristles—that is, by small thin, tapering ones—rather than by the rather heavy blunt ones of *repleta*.[4] In *melanogaster* and in *ananassae* the haplo-dot individuals are relatively fertile; in *simulans* as in *repleta* they are almost completely sterile. In *virilis* the fertility is so low that stocks are difficult to maintain. The gray pollinosity of the mesonotum that is affected by this chromosome in *repleta* and *hydei* is absent in the other species concerned, so this character could not show in them. The deepening of the groove between the mesonotum and scutellum, present in haplo-dot *repleta*, has not been recorded in these other species.

In *simulans* the haplo-dot individuals usually show a weakening or partial absence of the last section of the fourth vein. This character, which has not been observed in the other species, gains interest from the observation by MULLER and PONTECORVO (1942) that a *simulans* dot, transferred to *melanogaster* by an ingenious technique, acts as though it carried an incompletely dominant allele of cubitus-interruptus. It should be added, however, that my observations indicate that *melanogaster* cubitus-interruptus is fully recessive in diplo-dot F_1 hybrids between these species. I am inclined to suspect that the single transferred chromosome studied by MULLER and PONTECORVO may have undergone mutation at the time it was heavily X-rayed in their initial technique for transferring it to *melanogaster*.

In *melanogaster* (BRIDGES 1921) and in *ananassae* (KIKKAWA 1938) triplo-dot has been identified by cytological methods, but has not been found to show any clear-cut phenotypic difference from wild type—which would also be the case in *repleta* and *hydei* if, as in *melanogaster* and *ananassae*, one were unable to make use of the thoracic pattern.

It appears, then, that these four species resemble each other and differ at least from *repleta* (and probably also from *neorepleta* and *hydei*) in the phenotype of haplo-dot individuals. *Melanogaster*, *simulans*, and *ananassae* are closely related members of the subgenus Sophophora, while *virilis* and *repleta* are members of the subgenus Drosophila. I have, however, suggested (STURTEVANT 1942) that *virilis* is nearer to Sophophora than is *repleta;* the present result is consistent with that view.

The differences in phenotypic effects of the haplo-dot condition raise the question of the degree of homology between the dot-chromosomes of the species of Drosophila. Mutant genes located in the dot have been recorded in *affinis*, *ananassae*, *melanogaster*, *simulans*, and *pseudoobscura* of the subgenus Sophophora, and in *hydei* and *virilis* of the subgenus Drosophila. I have an

[4] It may be remarked here that in *melanogaster* most triploid strains of long standing come to have two, rather than three, dot-chromosomes. Such diplo-dot triploid individuals show definite suggestions of the Minute character; it is my impression that, with practice, one could learn to distinguish them from triplo-dot triploids by their slightly smaller and thinner bristles.

unpublished mutant type in *robusta*, also of the subgenus Drosophila. The references for the previously recorded types are given by STURTEVANT and NOVITSKI (1941).

The homology between the dots of *melanogaster* and *simulans* is clear. *Simulans* has produced a dominant allele of shaven, and a Minute-4 that resembled that of *melanogaster* and was shown to be a deficiency for *melanogaster* abdomen-rotatum. The experiments of MULLER and PONTECORVO (1942) with a *simulans* dot transferred to *melanogaster* furnish further and more detailed confirmation of the homology.

In the case of *anan.assae* the dot has become attached to part of X and is a small V rather than a dot at metaphase (KIKKAWA 1938). That it still contains the characteristic genes of the dot of other species (element F) is indicated by the occurrence of a dominant Shaven and by the phenotypes of haplo-dot and triplo-dot.

In *affinis* and *pseudoobscura* no haplo-dot or triplo-dot has been identified. The homology is indicated by the occurrence of grooveless in *pseudoobscura;* and of abdomen-rotatum, a possible allele of shaven, and a dominant that closely resembles the Cell of *melanogaster*,[5] in *affinis*.

These are all the mutant genes known in the element F of members of the subgenus Sophophora (other than *melanogaster*); since all of them can reasonably be supposed to parallel types known in *melanogaster*, it seems probable that the element has retained much the same genetic makeup and properties in all of them.

In the subgenus Drosophila the picture is not so clear. The one mutant known in the dot of *robusta* does not bear a close resemblance to any type known to me in any other species. The Extended of *hydei* would not be recognizable except by its recessive effect on the shape of the head and thorax in most species, and I know of no other type with those characteristics. The grooveless of *hydei* does agree very well with those of *melanogaster* and *pseudoobscura*, and the Cubitus-interruptus with the Gap of *virilis*. These two latter may also be compared to the cubitus-interruptus of *melanogaster* (which has a dominant allele), though with less confidence (see remarks above). In *virilis* there is also abdomen-rotatum that is a good parallel, and a reasonably satisfactory representative of shaven—an allele of which especially resembles the presumed shaven of *affinis*. There is also an eye mutant, glossy, that has been compared to *melanogaster* eyeless but which seems to me not at all a convincing parallel. The phenotype of haplo-dot virilis also resembles that of *melanogaster*, as indicated above, but that of *repleta* (and of *hydei?*) does not.

These comparisons may be taken as indicating that the dot of *virilis* is much like that of Sophophora, and that those of *repleta* and *hydei* (and perhaps of *robusta*) are somewhat less similar—though essential homology is still probable.

[5] The dominant in *affinis*, called Fused, was described by STURTEVANT (1940). Cell, discovered by GLASS, was described by BRIDGES and BREHME (1944).

SUMMARY

An X-ray induced dominant mutant in *D. repleta* is probably associated with a translocation between the dot and another autosome.

An aneuploid type produced by this translocation in interpreted as being haplo-dot.

A type phenotypically the opposite (as compared to wild type) is produced by *repleta-neorepleta* hybrids, and is interpreted as triplo-dot.

Triplo-dot × triplo-dot gives a more extreme type, probably tetra-dot.

Haplo dot is dark-colored, sterile, and has blunt but not Minute bristles; triplo-dot is light-colored and fertile; tetra-dot is still lighter and is sterile.

In *D. hydei* triplo-dot was detected by the use of the mutant Extended, and its composition was verified by cytological study.

Here also triplo-dot is lighter in color than wild type.

Preferential segregation occurs in triplo-dot *hydei;* it is more evident in males than in females.

A discussion of the available genetic data on the dot chromosome leads to the conclusion that it is similar in its properties in all the species of the genus studied, but among these is perhaps most different in *repleta* and *hydei*.

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GENETICS OF NATURAL POPULATIONS. XIII. RECOMBINATION AND VARIABILITY IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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INTRODUCTION

SPECIES of Drosophila show little variability as insect species go. Apart from a size variation which is mostly environmental, individuals that compose natural populations are as a rule uniform in appearance. Notwithstanding this external uniformity, natural populations of Drosophila contain a great store of genetic variants. The principal components of this store are recessive autosomal genes which, when homozygous, modify the viability, development rate, fertility, and other properties of their carriers. These genes are present in the populations mostly concealed in heterozygous condition (for further reference see DOBZHANSKY 1941; DOBZHANSKY and SPASSKY 1944; DUBININ 1946; IVES 1945; SPENCER 1944).

The concealed recessive variants are detected with the aid of experimental procedures, the essential part of which is obtaining individuals homozygous for chromosomes or chromosome sections which were carried in wild individuals in heterozygous state. Homozygotes display in their phenotype the effects of the recessives that were hidden in heterozygotes. The most frequently used version of this technique consists in crossing wild flies to laboratory strains which have some chromosomes marked by appropriate mutant genes and inverted sections. Flies are eventually obtained which carry a certain wild chromosome in duplicate, while the sibs of these flies carry the same chromosome only once. Comparison of the homozygotes and heterozygotes for the chromosome permits the detection of any recessive factors which this chromosome may carry.

It is evident that the above technique reveals only the net effects of homozygosis for whole chromosomes. How many genes may be involved in the production of these effects is not immediately apparent. Suppose, for example, that the homozygotes for a certain wild chromosome have a lower viability, a slower development rate, and some visible morphological peculiarity not present in their heterozygous sibs (such diversified effects of homozygosis for wild chromosomes are frequently met with in the actual experiments). What is the genetic basis of these changes? It is possible that the chromosome in question contains a gene for the low viability, another gene for the slow development, and still another for the morphological change. It is likewise possible that this chromosome carries a single gene with manifold effects responsible for the whole complex of characters. Finally, several or many genes may be involved in the production of each character, some of these genes having manifold effects and others apparently restricted fields of action. Experiments described in the present article have been devised to discriminate

between these possibilities. The importance of this problem from the standpoint of population genetics is considerable. The amount of potential variability stored in natural populations will be much greater if the properties of wild chromosomes are determined by multiple genes than if they depend upon single genes or very few genes. The amount of variability potentially available to the species determines its evolutionary plasticity. In the present experiments, the gene contents of three wild second chromosomes of *Drosophila pseudoobscura* from Mount San Jacinto, California, have been analyzed. It can be shown that these chromosomes carry complexes of genes which are capable of giving rise to a large amount of variability by recombination.

The work here reported has been supported in part by a grant from the CARNEGIE INSTITUTION OF WASHINGTON. It is a pleasant duty to acknowledge the help of MISS IRENE MARKREICH and of MR. BORIS SPASSKY in conducting the experiments.

THE BACKGROUND OF THE PRESENT INVESTIGATION

DOBZHANSKY, HOLZ, and SPASSKY (1942) have analyzed 302 wild second chromosomes of *Drosophila pseudoobscura* from three localities (Andreas Canyon, Piñon Flats, Keen Camp) on Mount San Jacinto, California. A very considerable amount of concealed variability was revealed by this analysis. The experimental methods, described and illustrated in the paper just referred to, are essentially as follows.

A wild male is crossed to females homozygous for the second chromosome recessive gene glass (*gl*). A single male from the offspring is outcrossed to females which carry in one of their second chromosomes the recessive *gl*, the dominant mutant gene Bare (*Ba*), and an inversion which causes a strong reduction of crossing over in the second chromosomes. This male must of necessity have one wild second chromosome and one second chromosome with the gene *gl*. In the progeny of the outcross, *Ba* females and males are selected and intercrossed. These *Ba* flies have a wild second, and a *Ba gl* inversion second chromosomes. In the offspring of the *Ba* flies, three classes of zygotes are formed: (1) wild type (non-Bare), homozygous for the wild second chromosome being tested; (2) Bare, heterozygous for the wild second and the *Ba gl* inversion second chromosomes; and (3) Bare glass, homozygous for the *Ba gl* inversion chromosome. The *Ba gl* homozygotes seldom survive as adults and are ignored in the counts. The theoretically expected ratio of the wild type to Bare flies is, therefore, 1:2, or 33.3 percent wild type to 66.7 percent Bare. This theoretical ratio, however, is realized only if the viability of individuals homozygous for the wild second chromosome and that of individuals heterozygous for the wild and for the *Ba gl* inversion second chromosomes are alike. In practice, this is far from always the case. The deviations from the expected ratios 33.3 percent wild to 66.7 percent Bare furnish a measure of the influence of homozygosis for the wild second chromosomes on the viability of the flies.

About 10 percent of the 302 wild second chromosomes tested contained

recessive lethal genes; the lethals are detected because in the crosses involving them the wild type class fails to appear, and the offspring of the *Ba gl* inversion females and males are Bar flies only. About 11 percent of the wild second chromosomes contain semilethals. A recessive semilethal is defined as a gene which destroys more than half of the homozygotes. The cultures involving semilethals consequently produce more than 0 but less than 16.7 percent of wild type individuals (the remainder, of course, being Bare). About 21 percent of the wild second chromosomes contain recessive genes which reduce the viability of the homozygotes to an extent not great enough to be classed as semilethals. The cultures with such "minus modifiers of the viability" produce more than 16.7, but significantly less than 33.3 percent, of wild-type individuals. Finally, a few of the chromosomes contain "plus modifiers of the viability" and give cultures which contain significantly more than 33.3 percent wild type.

The effects of homozygosis for wild second chromosomes on the duration of the development (development rate) of the flies are detected in a way similar in principle to the detection of the viability effects of the same chromosomes. The flies emerging from the pupae are classified and counted at frequent intervals (usually on alternate days) in the cultures in which the progenies of the wild/*Ba gl* inversion ♀ × wild/*Ba gl* inversion ♂ crosses are developing. Now, if the development rates of the wild type homozygotes and of the wild/*Ba gl* inversion heterozygotes are alike, then the ratios of wild:Bare flies in the successive counts will also be alike, within the limits of experimental errors. If, however, the wild chromosome carries a recessive gene, or genes, which slow down the development of homozygotes, then the proportions of wild type flies hatching in the cultures will be low in the first counts and will be relatively high in the later counts. This will, of course, be true regardless of the proportion of wild type individuals in the total count of the flies emerging in a given culture—in other words, regardless of the effects which a given second chromosome may have on the viability of the homozygotes. About 50 percent of the wild second chromosomes analyzed proved to contain recessive genes which, when homozygous, prolong the development of the flies; these genes are referred to as "minus modifiers of the development rate." A few chromosomes contain recessive "plus modifiers of the development rate": the homozygotes for these chromosomes hatch from the pupae significantly earlier than the Bare heterozygotes.

The detection of the recessive factors that produce externally visible morphological changes in the homozygotes requires no extended explanation. If the wild second chromosomes carry such factors, the non-Bare flies deviate from the wild type condition in their morphology.

The manifestation of recessive genes borne in the wild second chromosomes is frequently different in different environments. As shown by DOBZHANSKY and SPASSKY (1944), this environmental sensitivity is sometimes very high. Individuals homozygous for a certain wild chromosome, for example, may survive fairly well at some temperatures and may be little viable, or even com-

pletely lethal, at other temperatures. Apart from temperature, the manifestation of many genetic variants depends upon other culture conditions. Thus, the viability of homozygotes for some wild chromosomes may be relatively higher in cultures with low population densities than in more crowded cultures. Some homozygotes do, and others do not, respond to changes in the quality of the food on which the larvae develop. Chromosomes which in a certain environment produce similar effects may behave very differently in other environments.

The more the effects produced by homozygosis for wild chromosomes are investigated, the clearer it becomes that natural populations of *Drosophila pseudoobscura* contain a great variety of chromosomes with different gene contents. What is called the "normal" or "wild type" phenotype of the species is the average condition found in heterozygotes for *different* second, third, and fourth chromosomes. The number of these chromosomes with different gene contents present in natural populations is apparently high enough so that homozygotes for any one chromosome are in a minority. Furthermore, only few of the chromosomes may be called "normal," in the sense that individuals homozygous for them are indistinguishable from the average phenotype of the heterozygotes.

MATERIAL AND METHOD

Some 20 of the wild second chromosomes studied by DOBZHANSKY, HOLZ, and SPASSKY (1942) and by DOBZHANSKY and SPASSKY (1944) have been preserved in the laboratory in the form of balanced strains. In these strains, females and males carrying a given wild second chromosome and a *Ba gl* inversion second chromosome are selected and intercrossed in each generation. Since the inversion suppresses most of the crossing over, the wild chromosome is transmitted from generation to generation without change, except for the possible occurrence of mutations. Three wild second chromosomes have been picked for the present investigation. These are: chromosome No. 1015 from Andreas Canyon (to be referred to as "chromosome A"), No. 863 from Piñon Flats (chromosome B), and No. 975 from Andreas Canyon (chromosome C). A cytological examination in salivary gland cells showed all three chromosomes to have the normal gene arrangement. The other properties of these chromosomes are as follows (based on the data of DOBZHANSKY and SPASSKY 1944).

Chromosome A

Viability of homozygotes nearly normal at the temperature of 16½°C, semilethal at 21°, and completely lethal at 25½°. At temperatures at which some of the homozygotes survive, the development rate of the latter is considerably slower than that of the heterozygotes; the flies emerging from the pupae during two or more beginning days of hatching in a given culture are all Bare, wild type flies appearing only in later counts. Relatively more homozygotes survive in crowded than in underpopulated cultures.

Chromosome B

Viability of homozygotes normal or above normal at 16½° and 21°, normal

or slightly below normal at 25½°C. The results obtained in different cultures are uniform, regardless of the population density. Development rate is normal at all the temperatures tried.

Chromosome C

Viability of homozygotes normal or slightly above normal at 16½° and 21°, normal at 25½°. The results obtained in different cultures are uniform, regardless of the population density. Development rate is slightly slower in homo-

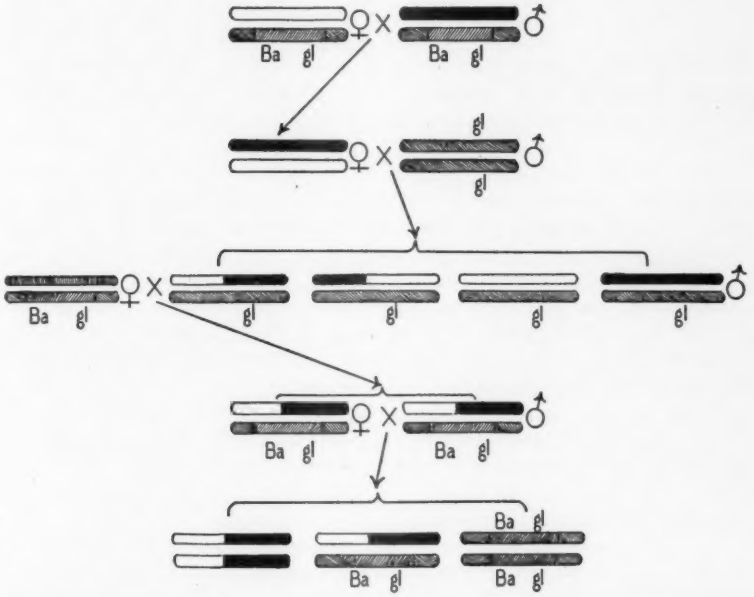

FIGURE 1.—The experimental procedure used to analyze the effects of recombination of genes in two wild second chromosomes (shown in white and black respectively). The laboratory chromosomes containing various marking genes or inverted sections are shown with the diagonal lines. *Ba*—the dominant Bare; *gl*—the recessive glass.

zygotes than in heterozygotes, but the difference is much less striking than in chromosome A.

To obtain crossing over between chromosomes A, B, and C, the experiments were arranged as shown in figure 1. Females carrying one of these chromosomes, say chromosome A (shown in figure 1 in white), and the balancing *Ba gl* inversion chromosome (diagonal lines), were crossed to males with chromosome B (black) and the same balancing chromosome. The wild type individuals obtained in the progeny all carry one chromosome A and one chromosome B. Such wild type females were crossed to males homozygous for

the recessive glass (*gl*). In the offspring of this cross, all flies carry a paternal chromosome with *gl* and a maternal wild chromosome. The maternal chromosome, however, may be an intact chromosome A, or an intact chromosome B, or it may be a chromosome compounded of sections of A and B by crossing over. It is, of course, impossible to tell by inspection of the phenotype of the fly which kind of a chromosome it carries; an examination of the properties of the chromosomes, however, is possible if flies homozygous for them are obtained.

A total of 100 wild/glass males (the third line in fig. 1) were selected and crossed individually to females having the *Ba gl* inversion chromosome. In the offspring, females and males showing the character Bare, but not showing glass, were selected and inbred. The composition of these flies is evidently wild/*Ba gl* inversion (fig. 1). From each culture, 12 females and an equal or greater number of males were taken, aged together for four days in a vial with food, and then transferred five times to fresh culture bottles at one day intervals. The five cultures containing the offspring of the same group of parents were placed in an incubator at 25½°C to develop. When the progeny in these cultures started to hatch, counts were made on alternate days. Just as in the above discussed experiments of DOBZHANSKY, HOLZ, and SPASSKY, the ratios of wild type to Bare flies obtained in the cultures can be used to measure the relative viabilities of homozygotes and heterozygotes for chromosomes A, B, and their crossover derivatives. The ratios obtained in the successive counts in the same cultures furnish a yardstick for measuring the relative development rates of the homo- and heterozygotes.

Experiments on recombination of genes in chromosomes A and C, and in B and C, were arranged as described above for the A–B combination, except that fewer chromosomes were tested in each case.

RECOMBINATION OF GENES IN THE CHROMOSOMES A AND B

A total of 97 chromosomes obtained in the offspring of females heterozygous for A and B chromosomes were successfully tested for their effects on the viability of homozygotes. The results obtained in the cultures raised at 25½°C are summarized in table 1, which shows the total numbers of the flies examined in the test generation (the last generation shown in fig. 1) as well as the percentages of wild type individuals among these flies. As indicated above, five cultures were raised for each chromosome (only three cultures, however, were fully counted for most of the lethal chromosomes). Although table 1 shows only the total counts in all the cultures involving each chromosome, the last column, marked "χ^2," gives the chi-squares that measure the degree of heterogeneity observed among the five cultures of each series. Each chi-square has four degrees of freedom. No chi-squares can be calculated for the lethals and extreme semilethals.

Inspection of table 1 shows that 37 out of the 97 chromosomes tested proved to be lethal when homozygous, as demonstrated by the failure of wild type individuals to appear in the cultures. Since one of the parental chromosomes (chromosome A) is also lethal to homozygotes at 25½°C (see above), these 37

chromosomes may be supposed to represent non-crossover A chromosomes; it will be shown below that some of them nevertheless are crossovers. Only four chromosomes (Nos. 3, 26, 73, and 91) produce normally viable homozygotes, as attested by the appearance of more than 30 percent of wild type individuals in the cultures. Homozygotes for four other chromosomes (Nos. 33, 44, 45, and 77) have viabilities only slightly below normal—they give between 25 and 30 percent of wild type flies in the cultures. Since the viability of homozygotes for the parental chromosome B is normal or subnormal (30.6 percent wild type among 1549 flies, see DOBZHANSKY and SPASSKY 1944, page 275), these eight chromosomes may be supposed to represent non-crossover chromosomes B. However, it is possible to show that at least two out of the eight possess properties which the original chromosome B did not possess. Namely, the homozygotes for chromosome No. 26 show a recessive visible effect, rather short and thin bristles. Homozygotes for chromosome No. 44 develop very slowly; only Bare flies emerge in the cultures during the first two to four days after the beginning of the hatching, while the late hatches consist of only or mostly wild type individuals. The homozygotes for chromosome B have normal bristles and develop at the same rate as heterozygotes. Barring mutation, the only way in which chromosomes Nos. 26 and 44 could have acquired new properties not present in chromosome B is by crossing over between chromosomes A and B. Therefore, only six out of 97 chromosomes tested may be said to be indistinguishable from the ancestral chromosome B.

Nine chromosomes (Nos. 13, 16, 20, 57, 59, 60, 67, 71, and 98, table 1) gave more than 16.7 percent but less than 25 percent of wild type flies in the cultures. In other words, the viability of homozygotes for these chromosomes is definitely below normal, but not low enough to be included in the class of semilethals. These chromosomes are obviously identical with neither the ancestral chromosome A nor B, and are derived from the latter by crossing over. Furthermore, these nine chromosomes are not alike among themselves. Homozygotes for chromosome No. 60 seem to develop faster than the heterozygotes ($\chi^2 = 5.03$, probability with two degrees of freedom about 0.08). Homozygotes and heterozygotes for chromosome No. 59 have the same development rates. The remaining seven chromosomes slow down the development of homozygotes very significantly. Chromosomes Nos. 13 and 71 give significantly different percentages of wild type individuals in different cultures of the same series, which indicates that the viability of homozygotes for these chromosomes is modified by some environmental agent that varies from culture to culture. In the case of chromosome No. 71, this agent is almost certainly the population density, since cultures which produced fewer flies gave higher proportions of wild type individuals than cultures with many flies, as follows:

Flies per culture	78	128	166	204	205
Percentage wild type	37.2	33.6	25.9	15.7	15.6

No such correlation with the population density is noticeable in the case of chromosome No. 13, although cultures with different numbers of flies have been obtained.

TABLE 1

Viability of homozygotes for each of the 97 second chromosomes obtained in the offspring of females carrying chromosomes A and B. Experiments at 25½°C.

CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2	CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2
1	641	2.5	—	48	1070	5.4	7.47
2	770	0.0	—	49	902	16.3	43.12
3	708	35.6	2.81	50	365	1.4	—
4	729	0.0	—	51	352	0.0	—
5	688	0.1	—	52	974	6.0	4.30
6	348	0.0	—	53	548	0.0	—
7	474	0.0	—	54	1006	3.0	44.85
8	547	0.0	—	55	366	0.0	—
9	711	4.4	26.49	56	1110	14.4	5.62
10	1079	7.2	13.96	57	1578	20.91	4.28
11	524	0.4	—	58	486	0.0	—
12	631	0.0	—	59	1049	21.0	0.23
13	1378	22.1	32.47	60	828	17.3	9.78
14	844	0.0	—	61	757	0.0	—
15	1113	6.8	13.82	62	592	5.1	—
16	1171	24.8	6.08	63	440	0.0	—
17	851	13.3	37.86	64	924	11.2	44.30
18	1385	0.0	—	65	860	13.0	24.44
19	729	0.0	—	66	838	0.7	—
20	709	22.8	5.25	67	622	19.8	5.10
21	347	1.4	—	68	811	5.5	13.62
22	861	13.2	5.90	69	442	5.4	—
23	699	10.7	8.84	70	695	4.6	—
24	1207	4.3	—	71	781	22.9	19.06
25	833	0.0	—	73	1163	36.3	3.09
26	1626	33.8	1.62	74	501	0.0	—
27	995	9.8	4.70	75	861	0.0	—
28	615	0.0	—	76	982	0.0	—
29	990	0.0	—	77	1007	28.3	10.82
30	519	0.0	—	78	1145	0.0	—
31	1533	0.0	—	79	636	16.0	11.83
32	1359	11.8	13.06	80	345	2.3	—
33	625	26.1	6.31	81	805	0.0	—
34	384	0.0	—	82	471	0.0	—
35	1312	5.6	23.46	83	659	0.0	—
36	463	0.0	—	84	536	2.4	—
37	1000	11.0	16.51	85	479	1.0	—
38	443	0.0	—	86	614	0.0	—
39	449	0.0	—	87	634	13.1	42.73
40	500	0.0	—	88	953	8.5	30.15
41	611	0.9	—	89	920	4.6	6.71
42	554	0.0	—	91	938	34.3	3.19
43	427	1.2	—	93	998	3.5	—
44	1015	27.8	2.92	94	1039	16.3	23.20
45	1087	29.2	3.16	95	811	3.9	—
46	643	0.0	—	96	787	13.8	86.69
47	643	0.0	—	97	829	4.6	13.12
				98	461	19.3	4.78
				99	462	0.0	—
				100	324	1.5	—

As many as 13 chromosomes gave percentages of wild type individuals ranging from ten to 16.7 percent, and ten chromosomes gave percentages below ten but above five per cent. Moderate recessive semilethals are evidently present in these 23 chromosomes. We may designate as extreme semilethals the 20 further chromosomes that gave less than five but more than zero percent wild type (table 1). Since neither chromosomes A nor B is semilethal at 25½°C (see above), these 43 chromosomes are crossovers between A and B. There is also no doubt that the semilethals do not represent a uniform group; rather, a collection of chromosomes with different properties is included in the semilethal class. This is confirmed by consideration of properties of these chromosomes other than their effects on the viability of the homozygotes.

The group of 13 moderate semilethals which gave between ten and 16.7 percent of wild type in the cultures may be considered first. Among them, three chromosomes (Nos. 17, 23, and 32) have development rates which do not differ perceptibly in homo- and heterozygotes. Nos. 37, 64, and 94 make homozygotes develop slightly but significantly slower than the corresponding heterozygotes. The other seven chromosomes produce more or less striking delays in the development of homozygotes. If we take chi-squares greater than 9.49 (which, with four degrees of freedom corresponds to a probability of about 0.05) to indicate significant heterogeneities among cultures of the same series, it follows that the viability of homozygotes for chromosomes Nos. 17, 32, 37, 49, 64, 65, 79, 87, 94, and 96 is sensitive to culture conditions, while that of the homozygotes for Nos. 22, 23, and 56 displays no such sensitivity (table 1). Examination of the data, however, discloses no significant correlations between the percentages of wild type individuals and the population densities in the cultures.

The ten semilethals that gave between five and ten percent of wild type individuals are an equally diversified group. Homozygotes for chromosomes Nos. 15, 27, and 69 have small wings that are usually bent arc-like over the back of the fly; the character is frequently asymmetrical, and some flies appear normal. Homozygotes and heterozygotes for chromosome No. 52 develop equally fast; Nos. 10, 68, and 88 slow down the development of homozygotes to a slight extent. Other chromosomes produce more or less striking delays in the development of the homozygotes. The percentages of wild type in different cultures testing the same chromosome are particularly variable for chromosomes Nos. 35, 52, 69, and 88. In no case, however, is there a suggestion of a correlation between this variability and the population density in the cultures. The 20 extreme semilethals are probably not a uniform group either, but very little can be said about them because only few flies homozygous for any given chromosome have been encountered.

It is obvious, therefore, that a considerable variety of chromosomes with different properties are produced as a result of crossing over between chromosomes A and B.

INFLUENCE OF LOW TEMPERATURE ON THE BEHAVIOR OF SOME LETHALS

The experiments described above have been carried at the temperature of

25½°C. In these experiments, 37 chromosomes completely lethal to homozygotes and 20 chromosomes that behaved as extreme semilethals were found in the progeny of females heterozygous for chromosomes A and B. Now, the ancestral chromosome A is known from the experiments of DOBZHANSKY and SPASSKY (1944) to behave very differently at different temperatures: it is lethal to homozygotes raised at 25½°, semilethal at 21°, while at 16½° these homozygotes survive nearly as well as do the heterozygotes. It was therefore

TABLE 2

Viability at 16½°C of homozygotes for second chromosomes obtained in the offspring of females carrying chromosomes A and B. These chromosomes were lethal or semilethal at 25½°C (cf. table 1).

CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2	CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2
1	834	34.9	2.03	46	751	32.8	4.78
2	911	30.2	24.33	47	888	34.4	6.47
4	1061	25.6	8.01	50	392	27.3	3.65
5	1457	33.3	2.15	51	1286	34.5	7.65
6	1295	30.5	8.56	53	709	33.9	0.89
7	780	26.0	7.90	55	595	22.2	2.73
8	1349	35.0	0.90	58	957	30.5	2.84
11	1378	23.7	5.30	61	1097	36.3	3.81
12	1088	30.9	5.77	62	277	34.7	2.25
14	1149	28.9	5.76	63	792	0.0	—
18	877	0.0	—	66	979	29.8	4.99
19	363	30.3	4.63	69	539	34.7	4.43
21	180	32.8	3.17	74	1006	35.9	8.89
25	1554	31.8	11.72	75	458	32.8	5.44
28	798	24.7	10.65	76	1488	25.2	50.51
29	1077	31.0	13.69	78	672	34.4	4.27
30	264	24.6	4.14	80	402	30.0	6.44
31	1202	21.7	19.19	81	1258	26.6	22.35
34	942	33.5	24.85	82	753	31.1	1.72
36	856	19.2	12.64	83	754	26.1	14.68
38	706	32.7	1.70	84	955	29.6	7.85
39	127	29.1	3.99	85	550	31.3	4.11
40	1321	31.4	4.36	86	1767	34.4	2.66
41	358	0.0	—	93	532	30.1	4.52
42	1070	29.2	11.59	95	514	34.1	5.76
43	730	29.5	1.42	99	483	33.9	9.51
				100	486	30.7	9.54

decided to test the behavior of the 37 lethal and 20 semilethal chromosomes at the low temperature, 16½°. The experimental technique remained the same as used for the experiments at the high temperature; the wild/*Ba gl* inversion flies were allowed to oviposit for about 24 hours at room temperature, five cultures being raised for each chromosome. The cultures then developed in a cold room at 16½°. The results are summarized in table 2, which is constructed like the previously discussed table 1.

Examination of table 2 shows that only three out of the 53 chromosomes tested (Nos. 18, 41, and 63) are completely lethal to homozygotes at 16½° as well as at 25½°. The appearance of these chromosomes is very interesting, since neither the ancestral chromosome A nor B are lethal or even semilethal at 16½°. The origin of the three lethal chromosomes must be due, disregarding mutation, to crossing over between chromosomes A and B. Chromosomes A and B evidently contain genes, or groups of genes, which taken separately are not lethal to homozygotes raised at 16½° but which become lethal when combined by crossing over. In view of their known origin by crossing over, chromosomes Nos. 18, 41, and 63 may be said to carry "synthetic lethals." No synthetic semilethals have appeared; the lowest proportion of wild type individuals given by any of the non-lethal chromosomes listed in table 2 is 19.2 per cent for chromosome No. 36, which is outside the semilethal range.

Among the 50 non-lethal chromosomes in table 2, six chromosomes gave between 19.2 and 25 percent of wild type individuals in the cultures, 12 chromosomes between 25 and 30 percent, 30 chromosomes between 30 and 35 percent, and two chromosomes (Nos. 61 and 74) 36.3 ± 1.4 percent and 35.9 ± 1.5 percent wild type respectively. Since all these chromosomes behaved as lethals or extreme semilethals at 25½°, their performance at 16½° is truly remarkable: a change of only 9°C causes a shift all the way from lethality to normal survival (fig. 2). This is, of course, what the ancestral chromosome A does as well; in the experiments of DOBZHANSKY and SPASSKY (1944) chromosome A homozygotes were lethal at 25½°, while at 16½° as many as 29.7 ± 1.4 percent wild type appeared in the cultures. What is important for us here is that the 50 chromosomes are not an uniform lot. Some of them make the homozygotes survive more and other less frequently than the ancestral chromosome A. They have evidently acquired new properties through crossing over between chromosomes A and B.

Individuals homozygous for chromosome A develop, at 16½°, appreciably more slowly than do the heterozygotes. It is, therefore, not unexpected that every one of the chromosomes listed in table 2 also slow down the development of homozygotes (the lethals must, of course, be disregarded since nothing can be told about the development rate of lethal homozygotes). The degree of the prolongation of the development is, however, not alike in all cases. Thus, the homozygotes for chromosomes Nos. 5, 14, and 55 seem to have their development slowed down more than in the homozygotes for other chromosomes.

The case of chromosome No. 76 is particularly interesting since in this case the decrease of the development rate is partly dominant. The hatching of the adult flies in the cultures involving this chromosome started about five days later than in other cultures made on the same date. The hatching continued in some of the cultures for as long as three weeks. The adult flies are small, paler than normal in coloration, and have relatively large and heavy bristles. At 25½° this chromosome is lethal to homozygotes, but the heterozygotes show only a slight delay in their development.

The last column in table 2 gives chi-squares that measure the heterogeneities among the five cultures raised to study each chromosome. Each of these chi-

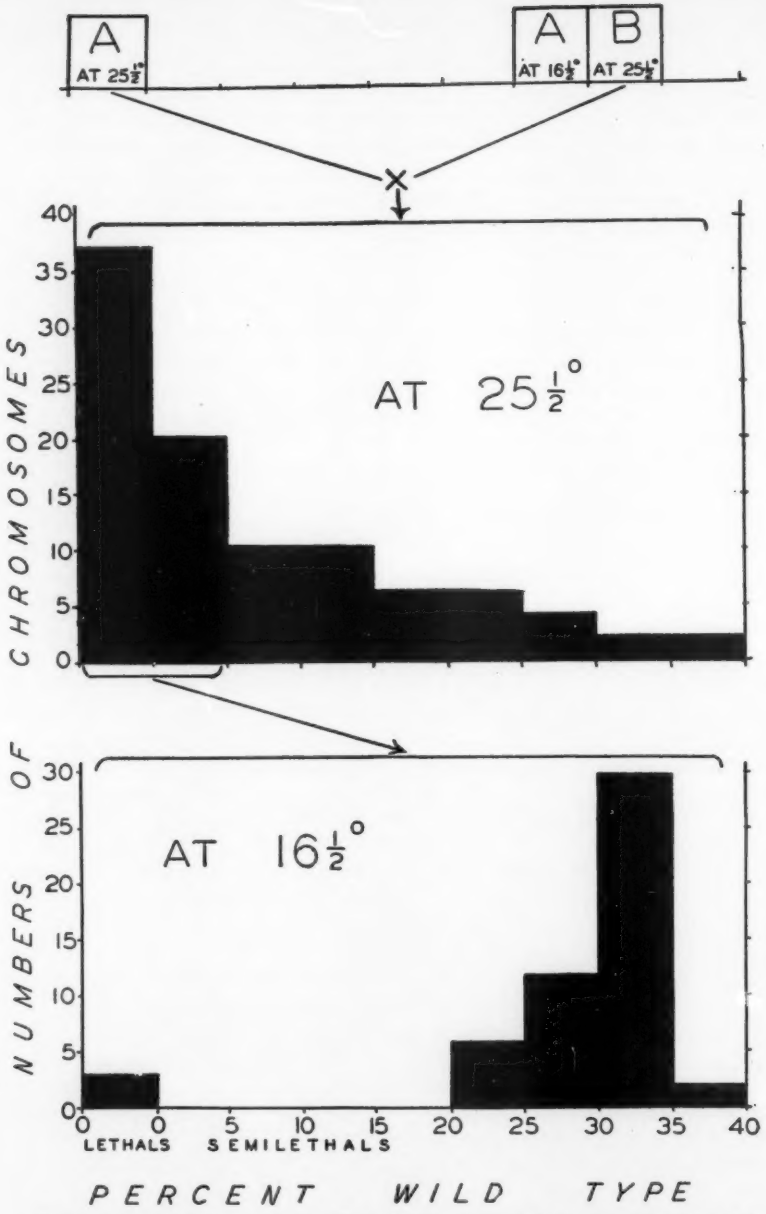

FIGURE 2.—Results of recombination of the viability genes carried in chromosomes A and B. The viability is measured through observing the proportions of wild type individuals in the cultures. Cultures containing between 30 and 35 percent wild type arise when the viability of homozygotes for a given chromosome is about equal to that of the heterozygotes. Failure of wild type individuals to appear in the cultures indicates that the chromosome involved is lethal to homozygotes.

squares has four degrees of freedom. Here as before, significant chi-squares indicate that the relative viability of homozygotes for a given chromosome is sensitive to differences in culture conditions that may occur among cultures made on successive days. Inspection of table 2 shows that most of the chi-squares are small. However, chromosomes Nos. 2, 31, 34, 76, 81, and 83 show very significant heterogeneities. The origin of these heterogeneities is obscure; the most obvious factor that could produce such differences among cultures, namely differences in population densities (different numbers of flies developing in different culture bottles), does not seem to be involved here.

TABLE 3

Viability at 25½°C of homozygotes for second chromosomes obtained in the offspring of females carrying chromosomes A and C.

CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2	CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2
1	801	0.0	—	16	1028	29.6	5.87
2	996	11.3	32.00	17	883	31.8	5.43
3	905	32.9	4.36	18	1055	0.0	—
4	1496	36.4	9.36	19	438	0.0	—
5	1069	17.1	2.69	20	1181	32.5	8.10
6	824	31.2	0.47	21	809	0.0	—
7	738	18.8	21.14	22	695	25.0	34.04
8	871	39.4	0.50	23	813	16.1	11.19
9	1148	7.5	5.45	24	996	31.4	9.50
10	1281	30.6	2.17	25	1021	32.8	5.81
11	1202	32.0	3.75	26	1095	34.1	2.31
12	837	4.3	—	27	1023	35.4	5.39
13	642	0.0	—	28	1148	33.9	1.52
14	1195	32.7	0.99	29	1104	30.3	0.97
15	783	0.0	—				

RECOMBINATION OF GENES IN CHROMOSOMES A AND C

The experiments on chromosomes A and C were arranged like those with the A-B combination, except that only 29 chromosomes obtained in the offspring of females heterozygous for A and C were tested. The data for the tests carried at 25½°C are summarized in table 3. It should be kept in mind in interpreting these data that chromosome A is lethal to homozygotes at 25½°, while homozygotes for chromosome C are normal in viability and have a development rate slightly but significantly slower than do the heterozygotes.

Only six of the chromosomes shown in table 3 are lethal when homozygous; these six chromosomes may represent the non-crossover chromosomes A. As many as 16 chromosomes gave percentages of wild type ranging from 29.6 per cent to 39.4 per cent; individuals homozygous for these chromosomes have, then viabilities approaching those of the heterozygotes. Some, but not all, of these 16 chromosomes may be non-crossover chromosomes C. That some of

them are in fact crossovers, is shown by heterogeneities too great to be explained by chance alone. Thus, as many as 39.4 ± 1.6 percent wild type appeared in the cultures involving chromosome No. 8, which indicates that homozygotes for this chromosome have viabilities greater than the heterozygotes. Furthermore, individuals homozygous for chromosomes Nos. 4, 10, 11, 14, 27, and 29 develop more slowly than do heterozygotes, while the remaining 10 chromosomes produce little or no prolongation of the development.

Chromosomes Nos. 9 and 12 are strong semilethals, while No. 2 is a moderate semilethal. Nos. 5, 7, and 23 are near the upper boundary of the semilethal range, while No. 22 decreases the viability of homozygotes to an extent far less than semilethal. All these chromosomes carry recombinations of genes contained in chromosomes A and C. Their effects on the development rate are heterogeneous. The development rates of the homo- and heterozygotes for chromosomes Nos. 2, 9, 12, 22, and 23 are alike or nearly so. A slight but significant delay occurs in homozygotes for No. 5, while the development of homozygotes for No. 7 is very strikingly slower than that of heterozygotes—the hatching of wild type individuals begins several days after Bare individuals have started to emerge from the pupae. Chromosomes Nos. 2, 7, 22, and 23 give heterogeneous results in different cultures (see the chi-squares in table 3). In Nos. 2 and 7 the heterogeneities seem to be uncorrelated with population densities in the cultures. There is an indication that low population densities are favorable for the survival of homozygotes for chromosome No. 23, as shown by the following figures:

Flies per culture	68	101	108	245	291
Percentage wild type	29.4	18.8	13.9	11.8	16.5

Conversely, homozygotes for chromosome No. 22 survive relatively better than do the heterozygotes in cultures with medium population densities. The data are as follows:

Flies per culture	40	74	178	191	212
Percentage wild type	0.0	4.1	28.1	36.7	24.1

Despite their lethality at $25\frac{1}{2}$°C, individuals homozygous for chromosome A survive nearly normally at $16\frac{1}{2}$° (see above). It may, then, be inferred that the lethal chromosomes shown in table 3 are non-crossover chromosomes A. The six lethals (Nos. 1, 13, 15, 18, 19, and 21) and the two semilethals (Nos. 9 and 12) shown in table 3 were, therefore, re-tested at $16\frac{1}{2}$°. A summary of the results appears in table 4.

As expected, all these chromosomes proved not to be lethal to homozygotes at $16\frac{1}{2}$°. Furthermore, every one of these chromosomes causes more or less striking delays in the development of the homozygotes as compared with heterozygotes. This is also a property of the ancestral chromosome A at $16\frac{1}{2}$°. And yet, it can be shown that some of the eight chromosomes listed in table 4 have properties which chromosome A did not possess. The viability of homozy-

gotes for chromosome No. 9 is lower than that of the other homozygotes, although a reference to table 3 will show that at 25½°C this chromosome behaved as a semilethal while other chromosomes (except No. 12) behaved as complete lethals. The homozygotes for No. 12 develop only slightly less rapidly than the corresponding heterozygotes, while the other homozygotes show more or less striking delays in their development.

RECOMBINATION OF GENES IN CHROMOSOMES B AND C

A great variety of chromosomes differing in viability, development rate, and other characters have been obtained by recombination of genes borne in

TABLE 4

Viability at 16½°C of homozygotes for second chromosomes obtained in the offspring of females carrying chromosomes A and C. These chromosomes were lethal or semilethal at 25½°C (cf. table 3).

CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2	CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2
1	1610	30.7	4.90	15	1068	33.3	6.31
9	783	18.9	10.86	18	988	32.2	3.99
12	1140	28.4	1.30	19	776	28.4	6.50
13	1468	29.6	2.84	21	952	26.5	15.31

chromosomes A and B or in A and C. The problem arises whether such results can be regarded typical for gene recombination in wild chromosomes in general. The ancestral chromosome A is, to be sure, exceptional in the wide range of behaviors which individuals homozygous for it display in different environments: they are nearly normally viable at 16½°, semilethal at 21°, and lethal at 25½°. Experiments on recombination of genes in chromosomes B and C should help to elucidate this problem; homozygotes for either of these chromosomes show normal or slightly below normal viabilities at 25½°, normal or slightly above normal at 16½°. Homo- and heterozygotes for chromosome B are alike in development rate; homozygotes for C develop slightly slower than the heterozygotes (cf. p. 273).

The experimental techniques used were the same as employed for the A-B and A-C combinations. The data for the tests carried at 25½° are summarized in table 5. Only 19 among the 33 chromosomes studied produced 30 or more percent of wild type flies in the cultures. Two other chromosomes (Nos. 2 and 23) produced 28.7 and 28.2 percent wild type respectively. The homozygotes for these 21 chromosomes may, then, be said to be about equal in viability to the corresponding heterozygotes, and to the homozygotes for the ancestral chromosomes B and C. The remaining 12 chromosomes are clearly different from both B and C. Among them, nine chromosomes gave between 21 and 27 percent wild type. These nine chromosomes produce slight but perceptible deleterious effects on the viability of homozygotes. Most remarkable are chro-

mosomes Nos. 1, 19, and 31, which produced 5.7 percent, 6.4 percent, and 10.3 percent wild type individuals respectively. These chromosomes fall, consequently, well within the semilethal range. Since neither chromosomes B nor C are semilethal to homozygotes, the three semilethal chromosomes may well be called "synthetic semilethals," analogous in their mode of origin to the synthetic lethals mentioned on p. 279. Recombination of genes in two wild chromosomes each of which makes the homozygotes normally viable may, then, give rise to chromosomes that act as semilethals.

TABLE 5

Viability at 25½°C of homozygotes for second chromosomes obtained in the offspring of females carrying chromosomes B and C.

CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2	CHROMOSOME NO.	FLIES EXAMINED	PERCENTAGE WILD TYPE	χ^2
1	681	5.7	2.24	17	993	32.1	6.21
2	1177	28.7	3.99	18	1092	22.9	0.58
3	125	38.4	7.86	19	597	6.4	2.66
4	1037	26.3	6.33	20	878	34.2	4.71
5	759	31.6	4.27	21	1253	31.0	2.23
6	582	34.0	5.88	22	702	33.3	5.47
7	846	31.8	1.80	23	865	28.2	9.55
8	441	22.5	6.28	24	695	21.2	3.48
9	1372	32.7	3.35	25	903	33.2	4.54
10	620	25.2	2.04	26	957	31.5	1.32
11	928	30.8	2.56	27	1334	26.1	4.76
12	1295	30.3	3.94	28	907	32.7	7.86
13	830	34.0	5.92	29	933	32.5	2.88
14	1016	26.2	8.29	30	1392	26.7	16.44
15	972	26.3	1.98	31	672	10.3	0.71
16	800	32.1	2.48	32	835	34.6	5.58
				33	351	33.9	8.27

Analysis of the data for the development rates shows only a moderate variability. Namely, nine chromosomes (Nos. 4, 5, 10, 11, 12, 14, 24, 26, and 28) produce statistically significant delays in the emergence of homozygotes. Homo- and heterozygotes for the other 24 chromosomes develop at equal rates. Among the nine chromosomes which do slow down the development rates of the homozygotes, all but No. 14 produce only slight delays, more or less comparable with that produced by the ancestral chromosome C. A greater, but still not striking, prolongation of the development is characteristic of chromosome No. 14.

Inspection of the chi-squares in table 5 shows that the results obtained in different cultures of the same series are mostly quite uniform. The only sizeable chi-square (16.44) is found in the data for chromosome No. 30. The probability that such or greater chi-square may occur by chance is about 0.003,

but since it occurred in only one out of 33 chromosomes it is probably not significant.

The viability of homozygotes for chromosomes that behaved at 25½°C as "synthetic semilethals" (Nos. 1, 19, and 31) was re-tested at 16½°C. The proportion of wild type flies observed in the cultures proved to be 6.4 percent, 31.8 percent, and 32.8 percent among 408, 1480, and 919 flies respectively. Chromosome No. 1 is, consequently, semilethal at both temperatures, while Nos. 19 and 31 are normally viable at 16½°C.

DISCUSSION

It has been shown that recombination of genes carried in only three second chromosomes derived from natural populations of Mount San Jacinto gives rise to a remarkable variety of chromosomes with different properties. These chromosomes range all the way from complete lethality in homozygotes, through various grades of semilethals, relatively mild deleterious modifications, and to chromosomes which make homozygotes equal or even slightly superior to the respective heterozygotes in viability. Individuals homozygous for some chromosomes survive relatively better than the heterozygotes in cultures with low population densities, others in more crowded cultures, while still others are not affected by population density variations. The influence on the development rate ranges from cases when homo- and heterozygotes develop equally fast, through more or less noticeable delays, and to striking retardations of the homozygotes. One chromosome causes a semi-dominant, instead of a recessive, modification of the development rate. Some chromosomes produce slight modifications of the morphology of the flies, although the chromosomes from which they were derived did not have such effects.

Although the three initial chromosomes used in our experiments can hardly be considered a fair sample of second chromosomes in natural populations of *Drosophila pseudoobscura*, it is clear from the data that a tremendous amount of variability is released by recombination of genes in wild chromosomes. If a population of a locality were to start with only few chromosomes, it would contain an imposing variety of chromosomes in the following generations. This may seem inconsistent with the relative uniformity of at least the external appearance of the flies which prevails in natural populations of most species of Drosophila, including *D. pseudoobscura*. The solution of the paradox is that the variability here involved is mostly concealed or potential rather than actually manifest in the phenotype. The variability is concealed because most of the mutant genes are recessive and present in populations in low concentrations, and also because wild chromosomes differ not in single genes but in gene complexes.[1] Concealed variability is released, as we have seen, through recom-

[1] MATHER (see MATHER 1943 and 1944 for further references) has introduced the convenient expression "polygenic character" (a polygenic character is defined by MATHER as one "whose inheritance is controlled by many genes each having an effect which is small compared with non-heritable variation") to describe the long known phenomenon variously called inheritance through multiple genes or multiple factors, modifiers, duplicate genes, blending inheritance, etc. MATHER also uses the noun "polygenes" to mean "linked combinations of the genes determining

bination of the gene complexes carried in the chromosomes of a population by crossing over, and through manifestation of recessive genes and gene complexes in homozygotes.

The existence of a store of potential variability underlying the much more limited variation actually observed in sexually reproducing and cross-fertilizing populations has been realized for a long time, although the tremendous magnitude of this store is only now becoming apparent. The idea of accumulation in populations of concealed recessive variants is clearly implicit in the now classic SHULL-JONES theory of causation of heterosis through covering-up of deleterious recessives by their dominant "normal" alleles (see JONES 1917, 1925, EAST and JONES 1919, SINGLETON 1941, and others). Following a clear statement of the problem of concealed variability by CHETVERIKOV (1926), many investigators in various countries, among whom DUBININ and his school must be prominently mentioned, have found that populations of species of Drosophila indeed carry a profusion of autosomal recessive mutants concealed by heterozygosis (reviews in DOBZHANSKY 1939, 1941).

DOBZHANSKY, HOLZ, and SPASSKY (1942) have pointed out that the magnitude of the store of concealed variability and of heterosis are functions of the genetic population structure. In species which reproduce by self-fertilization or close inbreeding, deleterious recessive mutants and deleterious recombination products of the gene complexes are eliminated by natural selection almost as rapidly as deleterious dominants. Little heterosis is observed in such species. The more prevalent is outbreeding in a species, the less frequently will any individual chromosome or gene complex become homozygous. Deleterious recessives will be eliminated much less rapidly than deleterious dominants, and heterosis will be more or less strongly pronounced. Extreme outbreeding will cause chromosomes and gene complexes to become homozygous so rarely that the welfare of the species will depend upon the effects of these gene complexes in heterozygotes with other gene complexes present in the same population, rather than upon their effects in homozygotes. Natural selection will, accordingly, favor those gene complexes which produce optimal adaptations

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quantitative variation". The gene complexes described in the present articles fit this definition of "polygenes." Since, however, a "polygenic character" is any character which is determined by many genes, regardless of whether these genes lie in the same or in different chromosomes, the noun "polygene" can only mean the sum total of the genes controlling a character which is "polygenic." To refer to individual genes entering in the determination of a polygenic character as "polygenes" would also be an etymological inconsistency. Furthermore, it must be emphasized that genes determining polygenic characters are not a separate category of genes, and that all transitions occur between polygenic characters and characters determined by single genes or few genes with large effects (MATHER's "oligogenes"). In one of his papers, MATHER (1944) conjectures that genes which determine polygenic characters may be concentrated in heterochromatic chromosome sections, but MATHER's evidence shows at most that some genes located in heterochromatin influence polygenic characters. Now, the history of genetics attests the futility of attempts to draw sharp distinction between the mechanisms of inheritance of qualitative and quantitative characters, even though the techniques of studying the extreme examples of these two classes are of necessity different.

in heterozygotes, largely regardless of their effects in homozygotes. The limiting case will be a species in which every chromosome is lethal when homozygous, and the degree of heterosis is, thus, at a maximum. Such a species is not known to exist, but, curiously enough, this limiting case is, as shown by MULLER as early as 1918, approached most closely by certain Oenotheras, but in them a singular chromosomal mechanism makes maintenance of heterosis compatible with self-pollination. MATHER (1941, 1943) refers to the effects of a chromosome (or of the genes it carries) in homozygotes as due to its "internal balance," while its effects in heterozygotes depend upon its "relational balance." MATHER'S conception of heterosis (1943), p. 52 is however quite different from ours.

The ability of the gene complexes carried in second chromosomes of wild *Drosophila pseudoobscura* to produce, through recombination, a great variety of new gene complexes disrupts the notions of "normal" or "wild type" chromosome, genotype, or phenotype. In general, these notions exist because of the reluctance of human mind to abandon the idea of a finite number of static prototypes underlying the unmanageable, and yet fascinating, multiformity of the living nature. In Drosophila and in other forms in which the naturally occurring individuals of a species tend to be uniform in appearance, it is, nevertheless, convenient for descriptive purposes to contrast mutant or aberrant individuals or strains with normal or wild type ones. In polymorphic species, such as man and maize, geneticists distinguish normal and mutant alleles only for loci in which one of the alleles occurs much more frequently than all others. In *D. pseudoobscura* and *D. persimilis*, the idea of "normal" gene arrangement in the third chromosome had to be abandoned because so many gene arrangements occur in natural populations that designation of one of them as "Standard" has been arbitrary. Now, the polygenic nature of the variation found in the second chromosome leaves little doubt in that no single gene complex is likely to be prevalent enough in the populations to justify its being called the "normal" condition.

The objection may be raised that the observed variety of gene complexes concerns their manifestation only in homozygotes, while they are mutually so adjusted as to produce a "normal" phenotype in naturally occurring heterozygotes. To a great extent this is undoubtedly true; the potential variability far exceeds the effective one. The genetic population size in *Drosophila pseudoobscura* (DOBZHANSKY and WRIGHT 1943) is however sufficiently small to make it probable that gene complexes sometimes do become homozygous in nature. More important is that natural populations of this species, although they do seem uniform in morphology, show important variation in physiological properties. DOBZHANSKY (1943) and WRIGHT and DOBZHANSKY (1946) have shown that, at least in some localities, third chromosomes with the "Standard" gene arrangement are favored during summer, while chromosomes with the "Chiricahua" arrangement are favored during spring season. The adaptive values of individuals with these gene arrangements are very different in some environments. It seems probable that the different gene arrangements acquire

different adaptive properties through association with different genes, rather than through position effects. The polygenic nature of the variation shows how such associations become established. A gene complex which produces a phenotype well adapted to some environments in which the species occurs in nature is, of course, favored by natural selection. However, crossing over tends to break up gene complexes regardless of their usefulness. Inversions that prevent break-ups of superior gene complexes acquire, consequently, positive selective values and increase in frequencies. Since heterozygotes are better adapted than homozygotes, several gene arrangements exist in a population in a moving equilibrium.

The theory of genic balance, according to which plus and minus modifiers for various characters occur intermingled in chromosomes, was developed by BRIDGES (1922, 1939). The bearing of this distribution on the effectiveness of natural selection was pointed out by GOODALE (1937), and more recently discussed in detail by MATHER (1941, 1942, 1943), MATHER and WIGAN (1942), and WIGAN and MATHER (1942). Numerous genes lying in the same chromosomes (gene complexes) provide superior storage facilities for the genetic raw materials of evolution than would be possible with a few major genes, and recombination of genes in such gene complexes makes possible great advances in rebuilding the organism in directions favored by artificial or natural selection. This, with or without the concomitant occurrence of mutations, accounts for the rather spectacular results of systematic selection obtained in *Drosophila melanogaster* by PAYNE (1918, 1921), ZELENY (1922), and MATHER and WIGAN (*loc. cit.*). The data reported in the present article have a bearing on the problem of selection, even though they involve no selection experiments in the usual sense of the term. Some of the chromosomes obtained through crossing over between the three ancestral wild chromosomes have properties very different from the latter. It is, therefore, possible to "select" products of recombination of the gene complexes that deviate greatly from the ancestral types, being completely outside the limits of variability of these ancestors. The "synthetic" lethals and semilethals, obtained by crossing over between chromosomes that are normally viable in homozygotes, provide the most striking illustration of the above statement.

SUMMARY

Three wild second chromosomes, derived from the population of *Drosophila pseudoobscura* of Mount San Jacinto, California, were chosen as material for experiments. One of these chromosomes is remarkable because individuals homozygous for it are highly sensitive to environmental influences: they survive nearly normally at 16½°, are semilethal at 21°, and completely lethal at 25½°C. Homozygotes for the other two chromosomes have viabilities about equal to those of the respective heterozygotes.

By means of appropriate crosses, females were produced that were heterozygous for any two of the above three chromosomes. The eggs of these females contained chromosomes some of which were identical with the ancestral ones

(non-crossovers) and some were new (crossovers). A sample of these chromosomes was analyzed for their effects in homozygous condition. A great variety of chromosomes with different properties were thus obtained. The viability of the homozygotes for some of the chromosomes is equal to or even slightly superior to that of the respective heterozygotes; other chromosomes act as recessive deleterious modifiers, still others as semilethals, and finally some act as complete lethals. Homozygotes for some chromosomes are relatively more viable than heterozygotes in crowded cultures, others are favored in cultures with low population densities, and still others are not sensitive to population density variations. Many of the chromosomes produce varying degrees of retardation of the development of the homo- as compared to the heterozygotes.

Particularly interesting is the appearance of "synthetic" lethal and semilethal chromosomes, which arise through crossing over between chromosomes lacking these properties. One chromosome has a dominant effect on the development rate of its carriers; no such effects were present in the ancestral chromosomes. At least two chromosomes have "synthetic" effects on the visible morphology of the flies.

It is concluded that chromosomes in natural populations of *Drosophila pseudoobscura* differ in complexes of linked genes most of which have small individual effects. These gene complexes afford a great store of potential genetic variability which may be released by crossing over and recombination, and then utilized for evolutionary changes. Genes with large individual effects also occur in wild chromosomes.

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INHERITANCE IN LADY BEETLES

IV. THE FORM OF THE CHITINOUS MALE GENITALIA IN CROSSES OF THE SPECIES HIPPODAMIA QUINQUESIGNATA AND H. CONVERGENS[1]

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BECAUSE of the reliance placed by taxonomists on the chitinous male genitalia of insects as distinguishing characters of species, information concerning the method of determination of their form assumes considerable importance. In a study of inheritance of color pattern in two species of Hippodamia, a cross between the species was effected, two F_2 families and two backcrosses to one of the species were obtained, and from these backcrosses or their descendants a series of matings with the F_2 and with both species was made. The course of the experiments was determined by the patterns involved, plus certain peculiarities of one line of descent and of lady-beetle inheritance in general (SHULL 1945). Information concerning the genitalia is in a sense a by-product, but perhaps more important than that relating to pattern. The only previous attempt to study the inheritance of the form of the genitalia in the coccinellids appears to be that of TIMBERLAKE (1919) who crossed *Hippodamia parenthesis* and *H. lunatomaculata*, and reported the genitalia to be intermediate. The experiments did not proceed beyond the F_1 generation. There may be some disagreement concerning the distinctness of these species, but the genitalia of the beetles TIMBERLAKE used must have been in some degree different.

THE CONTRASTS INVOLVED

The male genitalia of these beetles consist of a collar of distorted cylindrical form (the phallobase) with projections, and an unequal-armed U-shaped tube (the sipho or median lobe) whose distal end rests in or is thrust through the collar. The median projection from the phallobase (called the aedeagus) bears toward its tip in *H. quinquesignata* a sharp knifelike transverse keel or carina which slopes toward the base of the structure, as the visor over the vertical windshields of automobiles of the late twenties sloped downward. This keel is wanting in *H. convergens*. Beside the aedeagus are two movable appendages, the paramera or lateral lobes. While these are somewhat heavier in *H. quinquesignata*, there is little difference in their length in the two species. At the proximal end of the phallobase, there is a triangular projection which, in the absence of any name proposed by entomologists, is here called simply the basal triangle. This triangle differs in width and in height in the two species. From

[1] Contribution from the Department of Zoology, UNIVERSITY OF MICHIGAN. Aided by a gift from MRS. S. RALPH LAZRUS.

the ventral side of the phallobase projects a flat spatula-shaped structure, the tegminal strut or trabes, which is muscularly joined to the proximal end of the sipho. The width of this strut at its free end differs in the two species.

The sipho of *H. quinquesignata* is heavier than that of *H. convergens*. This difference is most marked near the distal end, where there is a considerable swelling in the former species, only a slight enlargement in the latter. Near this swelling in *H. convergens*, there arise dorsally two high, thin flaps (called so by TIMBERLAKE 1919) diverging somewhat like the elevated wings of a bird at the moment of alighting from flight. These flaps are reduced in *H. quinquesignata* to broadly based low arcs curved toward each other.

Choice of names for these structures is not to be interpreted as taking sides in a controversy over proper terminology; still less does it imply any conclusion concerning their homology with the structures of other beetles. The names are simply means of reference, and their significance is clearly shown in figure 1.

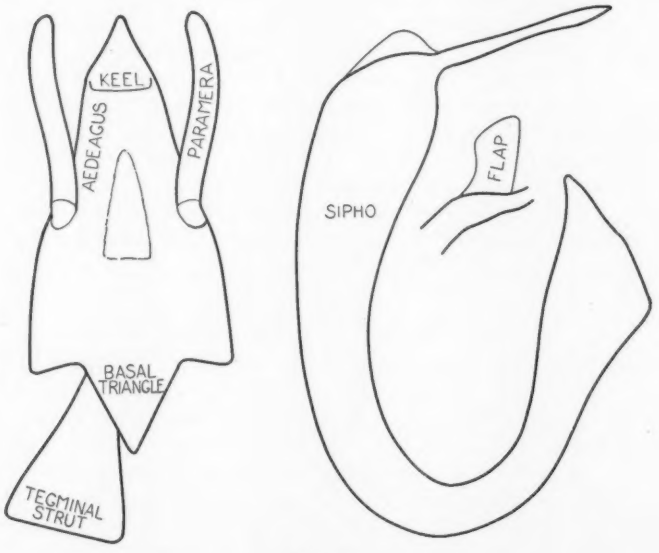

FIGURE 1

With the exception of the transverse keel of the aedeagus, which is present in one species, lacking in the other, the differences between the species are shown more exactly in table 1. Measurements have been made on *H. quinquesignata* from five sources, the first three of which contributed parents to the crosses described in this paper. From seven sources males of *H. convergens* were measured, and the first five of these groups contributed to the experi-

ments. The numbers refer to experiments. Two collections from California, from Yosemite National Park and Placerville, are recorded separately.

The length of the paramera is given in microns. At one stage in this study all paramera of the two species were averaged and found to differ in length by about one percent. While the width of the paramera probably differs, it would be difficult to make accurate comparison of such small measurements.

TABLE 1

Contrasts of male genitalia of Hippodamia quinquesignata and H. convergens.

SPP.	SOURCE	PARAMERA IN μ	HEIGHT OF FLAPS OF SIPHO	WIDTH OF SIPHO	WIDTH OF STRUT	BASE OF TRIANGLE	HEIGHT OF TRIANGLE
H.quin.	260	576± 5.4	.200±.008	.676±.014	.682±.015	.637±.028	.486±.032
	288	576±20.7	.207±.010	.757±.040	.683±.044	.576±.021	.471±.024
	Utah	613± 9.6	.164±.012	.703±.011	.639±.054	.608±.018	.491±.032
	Idaho	641±30.5	.149±.005	.703±.006	.712±.038	.595±.036	.526±.021
	258	607± 7.0	.173±.008	.711±.020	.610±.091	.604±.030	.513±.035
H. conv.	264	596±11.5	.456±.009	.351±.013	.595±.006	.315±.008	.334±.015
	Yos.	634± 5.6	.418±.004	.329±.003	.535±.010	.364±.012	.302±.008
	Colo.	603± 6.8	.417±.006	.318±.005	.505±.009	.339±.012	.283±.012
	331	575± 4.4	.421±.004	.306±.003	.480±.010	.354±.019	.348±.013
	329	570± 3.3	.429±.004	.320±.004	.502±.010	.313±.012	.368±.012
	330	575± 4.2	.426±.005	.331±.004	.450±.009	.362±.019	.320±.011
	Plac.	610± 6.2	.429±.005	.332±.004	.550±.012	.355±.013	.339±.012

The paramera of the two species are not therefore contrasted. They are used as standards of comparison for other measurements, in order to remove as nearly as possible one source of variation—namely, general size of body. Their advantages for this purpose lie in their size and the fact that, no matter how the genitalia were mounted, one of the paramera was always in position to be measured.

The other measurements are all fractions of the length of the paramera of the same individual. Comparison of the upper and lower parts of table 1 shows that the flaps of the sipho in *H. convergens* are more than twice as high, and the width of the sipho at its widest point is barely half as great, as in *H. quinquesignata*. These are the most marked differences. The width of the tegminal strut and both dimensions (width, or base, and height) of the basal triangle are all greater in *H. quinquesignata*. The shape (ratio of height to base) of the basal triangle was studied but gave indecisive results. Presence or absence of the transverse keel of the aedeagus must be considered qualitatively later.

COURSE OF THE EXPERIMENTS

The course of the experiments is indicated in figure 2. All females used as parents were taken from experiments and were known to be virgin. Males

were obtained from wild collections (Yosemite Park and Colorado) seven times, otherwise from experiments.

In general, aside from the original species cross (279) and the two F_2 families (291 and 293), the experiments were a succession of backcrosses to the two species types, so that they effected a progressive dilution of the genetic contribution of one species, a concentration of the contribution of the other. Accordingly, it is useful to indicate at the top of the columns of experiment

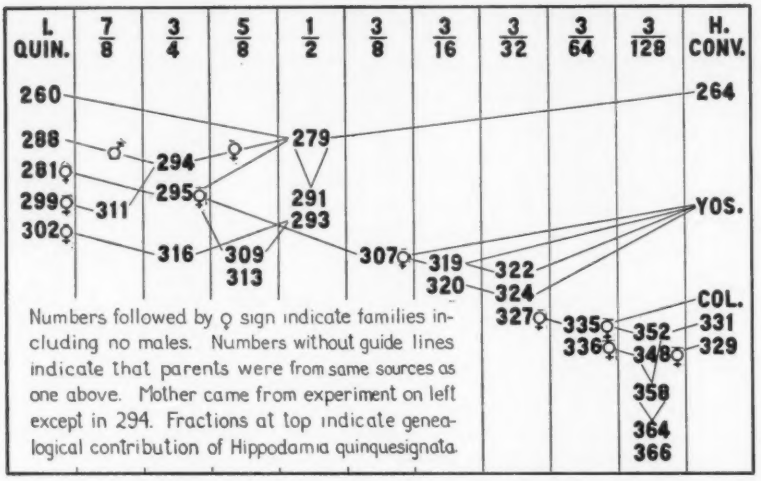

FIGURE 2

numbers, in purely genealogical terms, the fraction of the ancestry derived from one of the species by the families in that column. The fractions of *H. quinquesignata* are the ones shown.

The mother of any family came from the experiment to which the upward sloping guide line on the left points, except in 294 in which the source of the parents is reversed. An experiment number followed by the ♀ sign means that there were no males in that family, hence no measurements could be made.

INHERITANCE OF SIZE DIFFERENCES

The measurements of the contrasted characters fluctuate considerably even in groups which might be assumed to be genetically uniform. Accordingly, a male chosen for breeding might not, and usually did not, exhibit the mean measurements of the family or population from which he was taken. It does not seem feasible to use any such divergences in the interpretation of the results of the experiments; yet for the benefit of readers who may wish to try to use them, the characters of these male parents should be recorded. This is

done in table 2. The order of arrangement in this table is numerical to facilitate reference. Male parents not included in this table were lost. Several measurements are omitted because the relevant structure was lost, damaged, or distorted. All these males are included in the statistics of the families or collections from which they were derived.

The results of the experiments are given in table 3. The experiments are arranged according to the ancestral contributions of the two species—that is, it is the same as the arrangement in figure 2. It is believed that the results can best be interpreted from that arrangement.

The measurements given are means, with their standard errors. As before, paramera are recorded in microns, other measurements as fractions of the

TABLE 2

Measurements of genitalia of males used as parents in experiments.

FATHER OF	SOURCE	PARAMERA IN μ	HEIGHT OF FLAPS	WIDTH OF SIPHO	WIDTH OF STRUT	BASE OF TRIANGLE	HEIGHT OF TRIANGLE
279	264	588	.470	.368	.584	.335	.351
291	279	572	.311	.533	.667	.444	.411
294	288	525	.230	.794	.776	—	—
295	279	582	.328	.481	.710	.492	.328
311	294	601	.243	.550	.571	.466	.402
316	293	576	.276	.464	.613	.331	.315
320	Yos.	636	.425	.330	.520	.320	.250
322	Yos.	623	.383	.316	.536	.408	.306
324	Yos.	636	.410	.330	.550	—	—
327	Yos.	588	.422	.362	.562	.405	.324
335	Colo.	607	.424	.314	.524	.314	.346
336	Colo.	623	.398	.327	.561	.383	.306
348	329	595	.417	.299	.492	.353	.305
352	331	572	.433	.306	.506	.350	.300
358	352	541	—	—	.647	.500	.529
364	358	604	.405	.332	.568	.421	.421
366	358	572	.439	.306	.589	.472	.411

paramera. Standard errors are omitted at several places because only one measurement was made.

The columns of measurements in table 3 thus involve, from top to bottom, the consequences of a gradual dilution of the contribution of *H. quinquesignata* a gradual concentration of that of *H. convergens*. In the most marked contrasts (height of siphonic flaps and width of sipho) the gradient is quite obvious and fairly uniform. The F_1 generation (Exp. 279) and one of the F_2 families (293) are approximately midway between the two species as shown in table 1. The other F_2 family (291) is rather close to *H. quinquesignata* in the height of the flaps of the sipho, but is strictly intermediate in the width of the sipho. It should be stated that family 291 included only five males; 293 was little more

reliable with eight. From these middle values obtained from the F_1 and F_2 generations, the backcrosses and the other crosses genealogically equivalent to them lead to wider and wider siphones and lower and lower flaps as the *quinquesignata* ancestry is increased (upward in the table), and to narrower siphones and higher flaps as *H. convergens* is better represented (downward in table). The progeny of Experiment 324 have the *convergens* flaps, as perhaps

TABLE 3

Measurements of genitalia of males in all hybrid families.

FRACTION *quinque-signata*	EXP.	PARAMERA IN μ	HEIGHT OF FLAPS	WIDTH OF SIPHO	WIDTH OF STRUT	BASE OF TRIANGLE	HEIGHT OF TRIANGLE
7/8	311	564±24.7	.196±.022	.691±.053	.778±.009	.475±.034	.410±.064
3/4	294	555± 5.2	.230±.007	.586±.013	.618±.013	.550±.018	.396±.015
	316	572± 5.6	.218±.008	.602±.015	.629±.003	.469±.019	.434±.038
5/8	309	528	.319	.530	.723	.506	.422
	313	565± 6.8	.259±.008	.560±.010	.676±.012	.497±.012	.439±.007
1/2	279	579± 6.9	.308±.007	.492±.008	.667±.027	.463±.015	.385±.021
	291	559±13.0	.223±.007	.503±.016	.622±.033	.490±.037	.328±.026
	293	552± 8.0	.309±.011	.480±.010	.606±.025	.372±.023	.336±.018
3/16	319	604± 3.9	.393±.005	.415±.006	.625±.007	.479±.015	.393±.010
	320	617	.340	.479	.675	.433	.412
3/32	322	560± 5.0	.417±.007	.376±.006	.570±.012	.439±.025	.406±.016
	324	589± 1.8	.430±.005	.370±.004	.544±.010	.367±.018	.353±.016
3/128	352	541	.453	.329	.638±.006	.397±.073	.470±.041
	358	570± 5.2	.427±.004	.336±.004	.545±.008	.366±.011	.403±.010
	364	552±10.5	.429±.010	.352±.013	.568±.027	.385±.022	.522±.035
	366	566± 9.0	.442±.034	.339±.009	.518±.023	.349±.013	.413±.021

also do the offspring of Experiment 322; but neither of these families has as narrow siphones as does *H. convergens*.

The other three characters (involving the strut and basal triangle) give disappointing results. Though the two species, both in the wild and in the laboratory, differ in these characters (see table 1), their hybrids show great irregularity. The width of the triangle at its base is in general intermediate in F_1 and F_2, and shows a slight decline as *H. convergens* is approached; but the gradient toward *H. quinquesignata* is scarcely observable. The height of the triangle in F_1 is intermediate, but F_2 reverts practically to *H. convergens*, and the other crosses are very irregular, with those approaching the two species not certainly different from one another.

It seems impossible to explain the measurements of the tegminal strut and

the basal triangle on any satisfactory genetic basis. The interpretations of quantitative characters to be offered later refer, therefore, only to the characters of the sipho.

KEEL OF AEDEAGUS

It was not practicable to make any significant measurement of the sloping keel on the aedeagus. It is possible that a vertical longitudinal section through it would have revealed some measurable difference in the extent to which it rises from the general surface; but in surface view, if present at all, it appeared in most families to be fully developed.

Obvious reductions of the keel are found in Experiments 319 and 322. In Experiment 319, it is slightly developed in 11 males, lacking in 15. In Experiment 322, it is very slightly developed in three, wanting in eight. In Experiment 324, genealogically the equivalent of 322, the keel was entirely lacking. In 320, the genealogical equivalent of 319, the one male obtained plainly had the keel. In all experiments to the left of these four in figure 2, the keel was present and apparently well developed; in all to the right of them it was absent.

INTERPRETATION OF RESULTS

The gradations of the width of the sipho and the height of the flaps borne by it suggest that the genetic basis of these characters is a group of similar nondominant epistatic genes. Since the irregularities of the tegminal strut and basal triangle, which are to be ignored, present no greater difficulties for this explanation than for others, it is tentatively adopted.

The principal question to answer concerning such a system of genes is how many cooperating genes there are. A formula for determining the number was devised by WRIGHT and published by CASTLE (1921). This formula rests on the expected greater variability of the F_2 generation as compared with F_1. Unfortunately this formula leads to impossible results in the beetle experiments, for the standard deviation of the flaps of one of the F_2 families (291) is less than that of F_1, and the standard deviation of the other F_2 family (293) is so little greater than that of F_1 that the number of genes indicated by the formula is less than one. There are possible reasons for the failure of the formula. One of the requirements for validity of WRIGHT's formula is a sizable F_2 generation. This requirement is not met; as stated earlier, there were only five males in family 291, eight in 293. Another requirement is that the two species be homozygous, so that all members of F_1 (279) have the same genotype. The fact that the two F_2 families differed in the height of their siphonic flaps ($0.223 \pm .007$ in one, $0.309 \pm .011$ in the other) could mean that their respective parents were genotypically different. The same argument does not apply to the width of the sipho, in which the F_2 families are very much alike and like the F_1.

None of the other measurements leads to any better results with WRIGHT'S formula. There remains the rule-of-thumb method of estimating the number of genes involved, which will be followed. The fact that the siphonic flaps in family 324 are as high as those of *H. convergens* (table 3), while those of 322

may not be quite as high as in *convergens*, indicates that the *H. quinquesignata* genes had almost disappeared in their mothers, which were drawn from Experiments 320 and 319, respectively (see fig. 2). It would be a minimum assumption that the mother of 322 was heterozygous for one of the *quinquesignata* genes, and that the mother of 324 was strictly *convergens* with respect to these genes. On this basis, the mothers of 319 and 320 would best be regarded as heterozygous for one pair of genes. If family 307 as a whole is like these two members of it in being heterozygous for one pair, then family 295 should carry two genes. From 295 it is possible to jump to the pure *H. quinquesignata* and, in order to assign it an even number so as to constitute pairs, regard it as possessing four (two pairs) of the pertinent genes.

The F_1 generation (279) should thus have two (probably one of each pair), the F_2 families (291, 293) a variable number but averaging two. It is not necessary to postulate the number of contrasted genes for each of the backcross and other hybrid generations. The mean height of their siphonic flaps fits rather well the assumptions already made, particularly if the genes are not equal.

A similar argument may be drawn from the width of the sipho. The nearest families to *H. convergens* to show any certain influence of the *quinquesignata* genes are 322 and 324. Both of these families, not just one of them as in the height of the flaps, show *quinquesignata* influence. If they be assumed to have one *quinquesignata* gene, then 319 and 320 should, as a mean assumption, have two. Family 307 should have four, 295 should have eight, and 281 (pure *H. quinquesignata*) ten, (possibly 12). It is within the realm of lower probability, however, that somewhere in the chain of chances, the same experimental results could be obtained from a smaller number of contrasted genes. Thus, there is one chance in four that the mothers of 322 and 324 would both be heterozygous for one *quinquesignata* gene, even if 319 and 320 should likewise be heterozygous for only one such gene. This eventuality would lead to the conclusion that 281 had probably three pairs. This point is raised because of a later discussion as to whether the same genes might determine both the width of the sipho and the height of the flaps which arise from it.

With respect to the transverse keel of the aedeagus, the pertinent facts have been stated. Family 324 has none, but in the one male of 320 it is present. Family 322 shows a slight development of the keel in less than half its males, as does also 319, from which 322 was partly derived. Family 320 must have included at least one beetle (the male) which possessed at least one *quinquesignata* gene, but another (the mother of 324) which was strictly *convergens*. Family 319 must have included some individuals (the mother of 322, for example) which were heterozygous for one *quinquesignata* gene, but others (the keelless males among them) which were strictly *convergens*. From this constitution of 319 and 320, it is to be inferred that 307 is heterozygous for one pair of *quinquesignata* genes, that 295 possessed two such genes, and 281 probably two pairs of them.

The most direct arguments thus lead to the conclusion that two of the

characters distinguishing the species are differentiated through two pairs of genes, the third through five pairs. In each of the chains of random events, however, there is room for fluctuation such that the number of pairs of genes could be three for each character. It is permissible to inquire, therefore, whether the same genes may be differentiating all of them.

CORRELATION OF FLAPS WITH WIDTH OF SIPHO

Since in the two contrasted species wide sipho goes with low flaps, narrow sipho with high flaps, any genetic variability of these structures in the hybrids should show a negative correlation if the same genes determine both characters, no correlation if wholly different groups of genes are responsible for them. How decisive this fact might be would depend on the nature of any nongenetic variability to which the siphonic width and height of flaps are subject. In view of the possibility that the identity or distinctness of the genes involved might thus be indicated, the coefficients of correlation of width of sipho and height of flaps have been computed for all experiments or collections of beetles except those in which only one male was available. These coefficients are shown in table 4. Because of the notorious unreliability of coefficients based on very small numbers, the number of males measured is indicated. These numbers include all the males which were obtained except in some of the wild collections.

Though they are quite variable, the coefficients of correlation are all positive for beetles collected in nature or bred from them without crossing. If it may be assumed that members of the pure species are isogenic with respect to the characters involved, this positive correlation presumably means that nongenetic developmental factors act on both of them, in the long run, in the same direction. The measurements compared are not absolute sizes; if they were the presence of large and small individuals would create the observed correlation. They are instead relative measurements, fractions of the paramera. Any sipho which is exceptionally wide, relative to the paramera of the same individual, tends somewhat to have high flaps. This relation is hardly surprising, since the flaps rest on the sipho at its widest point, and any growth factors affecting this region could—perhaps would necessarily—tend to affect both measurements.

In the hybrid families the correlation is sometimes positive, sometimes negative, sometimes too small to mean anything regardless of sign. Some order is found in this variation, however. The families which are genealogically near the two parent species show positive correlation. Thus, Experiment 311, which is mostly *H. quinquesignata*, is positive; the extreme degree of correlation here (0.98) must be accidental, which it could easily be since it is based on only four individuals. Likewise, Experiments 322, 324, 358, 364, and 366, which are genealogically near *H. convergens*, show positive correlation, though of such low value in one of them as to be doubtful. The F_1 family (279) has positive correlation, the F_2 families (291, 293) negative. In the other hybrid generations the correlation is negative.

The probable interpretation of these facts is that genetic factors common to

the sipho and the flaps resting on it lead to negative correlation, and nongenetic developmental factors, or genes which do not constitute a difference between the species, produce a moderate positive correlation. In families or collections which are isogenic or nearly so, the nongenetic factors produce all or most of the variation, and correlation is positive. In families where segregation of genes can occur, there must be a conflict between the genetic and nongenetic variation resulting in low positive or outright negative correlation. The families from 322 on to the end of table 4 would, on this interpretation, be nearly *H. convergens;* 311 would be almost *H. quinquesignata.* The F_1 generation (279) would, if each species is homozygous, be isogenic, hence its correlation would be dependent on nongenetic factors; in the F_2 families (291, 293), however, there should be genetic as well as nongenetic variability. The positive correlation in F_1, negative in F_2, is in accord with this interpretation.

TABLE 4

Correlation between width of sipho and height of siphonic flaps. Abbreviated wild sources are Colorado, Yosemite Park, and Placerville, California. N, number of males; r, coefficient of correlation.

FRACTION *H. quinquesignata*	EXP. OR WILD SOURCE	N	r	FRACTION *H. quinquesignata*	EXP. OR WILD SOURCE	N	r
All	260	8	+0.31	3/16	319	26	−0.03
	288	3	+0.74		320	1	—
	Utah	10	+0.16	3/32	322	11	+0.04
	Idaho	3	+0.35		324	18	+0.22
	258	8	+0.11	3/128	352	1	—
7/8	311	4	+0.98		358	34	+0.26
3/4	294	15	−0.42		364	3	+0.73
	316	4	−0.90		366	3	+0.55
5/8	309	1		None	264	3	+0.35
	313	6	−0.06		Yos.	29	+0.51
1/2	279	9	+0.32		Colo.	19	+0.66
	291	5	−0.17		331	11	+0.28
	293	8	−0.61		329	22	+0.54
					330	12	+0.78
					Plac.	25	+0.21

Whether all, or only some, of the genes are common to the sipho and its flaps is a question which the correlation is hardly reliable enough to answer. Either assumption could be made with modest support. It is likewise possible, so far as the correlations show, to assume either that the species are strictly homozygous for the genitalia genes or that there is some genetic variability in either or both of them. The chief indication of genetic variability within the

species is the difference between the two F_2 families (291 and 293), to which attention has already been called.

FAMILIES DIVIDED WITH RESPECT TO KEEL

Correlation between the keel of the aedeagus and the characters of the sipho cannot be ascertained in the usual way, since the keel was not measurable. There are two families, however, in which some members have a slightly developed keel and others lack it. In Experiment 319, the number of each kind is large enough to justify a comparison of their other characters. This is done in table 5. It is there shown that individuals with the keel had lower flaps and wider sipho than did those without the keel. The differences are great enough to have a probability of over 0.97 of being real. They are in the direction that would be expected if the genetic basis of the keel were also partly responsible for the height of the siphonic flaps and the width of the sipho.

To complete the comparison of the beetles with and without the keel, the correlation between height of flaps and width of sipho has been computed for

TABLE 5

Contrast of height of siphonic flaps and width of sipho in males with and without an aedeagal keel in Experiment 319.

PRESENCE OR ABSENCE OF KEEL	NUMBER OF INDIVIDUALS	HEIGHT OF FLAPS	WIDTH OF SIPHO	CORRELATION OF WIDTH WITH FLAPS
With Keel	11	.381 ± .008	.428 ± .009	+.0054
Without Keel	15	.402 ± .006	.405 ± .008	+.2431
Difference		.021 ± .010	.023 ± .012	
Probability		.986	.971	

each group separately. The coefficients are shown in the last column of table 5. There is no demonstrated correlation in the group with keels, a small positive correlation among those without keels. The latter result is more or less in harmony with the assumption made earlier—namely, that the fifteen beetles without keel in Experiment 319 are strictly *H. convergens* in their genitalia genes—that is, that they have no genetic basis for variability. If the difference between these two correlations is significant, and if the proper interpretation has been put upon correlation in these structures, then there should be some genetic variability within the group of beetles with the keel. This conclusion would be at variance with the one arrived at earlier—namely, that these beetles with keels have each just one *quinquesignata* gene for each of two features of the genitalia—necessarily the same gene in each if their mother were, as postulated, heterozygous for only one pair of the differentiating genes.

There is too much uncertainty about the validity of the comparison of correlations to render further speculation regarding its meaning of any value. At best the number of genes involved and the extent to which the same genes affect the several characters, are still in doubt.

LOCK-AND-KEY HYPOTHESIS

The results here described may have some bearing on the view of many evolutionists that insect species are reproductively isolated from one another by the mechanical incompatibility of the genitalia of the two sexes in different species. The idea goes back to DUFOUR (1844), who regarded the male genitalia as "destined to be adapted to the external sexual parts of the female for accomplishment of the copulative act," and as the "guaranty of the conservation of the type, the safeguard of the legitimacy of the species." It has been expressed many times since.

In these two species of Hippodamia copulation was frequently observed in confined interspecific pairs. Moderate restlessness was exhibited by some of these pairs, which might result from some degree of lack of correspondence. One might suppose that the high siphonic flaps of *H. convergens* would be an obstacle or source of irritation in the female *H. quinquesignata*, though it is difficult to see any feature of the female which would make them less of an obstacle or irritation in *convergens* itself. The fact that copulation was frequent but that only one of three interspecific pairs produced offspring—plus the not infrequent occurrence but usual ineffectiveness of copulation between species of Hippodamia in nature—seems to indicate that the reproductive isolation rests mostly on something else than the form of the genitalia.

SUMMARY

The male genitalia of the two species differ in that a transverse keel of the aedeagus present in *H. quinquesignata* is lacking in *H. convergens*, and that the former species has a wider sipho, lower siphonic flaps, wider tegminal strut, and larger basal triangle than does the latter.

The keel, width of sipho, and height of flaps are inherited in polygenic fashion; the other characters do not fall into a readily recognizable genetic scheme.

The crosses involving the species were made in such a way as to produce a gradual dilution of the contribution of one species, a gradual concentration of that of the other. The points in this gradient at which the characters of one species disappeared lead to the conclusion that the siphonic flaps and the aedeagal keel may be differentiated in the two species each by two pairs of genes, the width of the sipho by five pairs, with a distinct possibility that the number could be three pairs for each of them.

Positive correlation in the pure species and in families with little or no genetic variability, and reduced or negative correlation in families in which genetic variability was to be expected, lead to the conclusion that at least part of the genetic basis is common to the flaps and width of sipho.

Members of one family in which some had the keel, others lacked it, showed differences in the sipho and flaps in the two groups which indicate that the genetic basis of the keel is partly common to the siphonic characters. The data are inadequate to show how extensive the common genetic basis of the keel, flaps, and width of sipho may be. Lack of correlation between width of sipho

and height of flaps in the group possessing the keel could be interpreted to indicate some genetic variability in that group, which would require more genes than are postulated above.

The inequality of two small F_2 families suggests, but does not prove, that there may be some genetic variability in the height of the siphonic flaps within one or both of the species.

The frequent interspecific copulations in experiments, and their occasional occurrence in nature, together with the obvious infrequency of successful species crosses, indicate that the form of the external genitalia is not an important factor in the reproductive isolation of the species.

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INCIPIENT INTRASPECIFIC ISOLATING MECHANISMS IN RANA PIPIENS[1]

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THE frog, *Rana pipiens* Schreber, in common with many wide ranging species, is highly variable. Throughout its range of distribution a multitude of local races have developed. Variable species, such as *Rana pipiens*, are of importance as material for the study of the process of speciation. In the dim outline of the *modus operandi* of this process several features seem of prime importance. First, there must be an increase in the frequency of certain genes in some portion of the population. Second, a method of maintaining the distinction of the newly diverged portion of the original population is necessary if gene differentiation is to result in more than intraspecific variability. The methods by which the maintenance of genetic diversity is accomplished are known as "isolating mechanisms." If effective reproductive isolating mechanisms become established between the two differentiated portions of the population, gene exchange will be prevented. The development of reproductive isolating mechanisms is, therefore, a crucial step in speciation. It is with this problem that the present paper is primarily concerned.

Regional divergences have obviously occurred in *Rana pipiens*, and some experiments on hybridization between individuals from Florida and Vermont indicated that these two populations were partially isolated by hybrid inviability (MOORE 1942[2]). These observations appeared to indicate that some populations of the *Rana pipiens* complex are nearing the species stage of divergence.

The crosses just mentioned were made primarily to study the influence of the sperm nucleus on rate of development. In the two types of hybrids, Vermont ♀ × Florida ♂ and Florida ♀ × Vermont ♂, the rate of development was maternal in early stages, but in the late gastrula and neural plate stages the hybrids fell behind the controls. The Vermont ♀ × Florida ♂ embryos had greatly enlarged heads and defective circulatory systems. In many the heart failed to beat, and those with a functioning heart usually had defective gill circulation. A small percentage of these hybrids was sufficiently normal to reach metamorphosis, but the majority died shortly after hatching. The reciprocal cross, Florida ♀ × Vermont ♂, resulted in embryos with a different

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[2] In this first paper the accepted taxonomic designation for races of *Rana pipiens* was employed pending further study. Individuals from southern Florida were referred to as *Rana sphenocephala* and the Vermont form as *Rana pipiens*. Subsequently it was shown that the diagnostic characters of these two "species" were not valid and that all meadow frogs of eastern North America should be known as *Rana pipiens*. (MOORE 1944).

type of abnormality. Instead of showing enlarged heads, the anterior region was much reduced. The circulatory system appeared to function normally, but in crosses of this type the survival is low.

It was clear from these results that isolation through hybrid inviability has reached a high intensity between the Vermont and Florida races of *Rana pipiens*. Gene exchange would be slight even if the two populations were sympatric. It was equally apparent that this method of isolation was of no importance under natural conditions. The possibility of a Vermont *pipiens* crossing with a Florida one would be extremely remote without the interference of a zoologist. However, if adjacent populations would behave in this manner, hybrid inviability would be an effective isolating mechanism. Consequently, an attempt was made to cross individuals from as many populations as possible to determine the extent to which hybrid inviability acts as an isolating mechanism. A preliminary report of some of these experiments has been given (MOORE 1943).

MATERIALS AND METHODS

Rana pipiens from the following localities have been used in hybridization experiments: Alburg, Vermont; Oshkosh, Wisconsin; Mt. Ephraim, New Jersey; Tulsa, Oklahoma; Chalmette, Louisiana; Monahans, Texas; Ocala, Florida (central part of state); and Englewood, Florida (southern part of state). I am indebted to DR. A. P. BLAIR for the Oklahoma and Texas material. Frogs from the remaining localities were secured from dealers who kindly supplied information concerning the source of the material.

Eggs were obtained by the standard pituitary injection method. They were artificially fertilized with a sperm suspension made from the testes of single males. The developing eggs were raised at constant temperatures in water baths or incubators.

In most experiments males and females from several localities were used. The embryos were examined at frequent intervals during early development. A special effort was made to compare rates of development, since deviation from the normal in this respect is probably the most sensitive indicator of any ill effects due to hybridization. A close comparison of hybrids and controls was also made to detect any indications of morphological abnormality in the former.

Development is expressed in terms of Pollister's Stages (POLLISTER and MOORE 1937). The more important of these are as follows: Stage 3, first cleavage; Stage 10, beginning of gastrulation; Stage 11, blastopore 180 degrees; Stage 12, blastopore complete; Stage 13, neural plate; Stage 14, neural folds; Stage 16, neurula; Stage 17, tailbud; Stage 18, muscular contraction begins; Stage 19, heart beat begins; Stage 20, gill circulation begins. Frequently the Stages are recorded as early, middle or late (as 10E, 14L, etc.), which introduces a further refinement. In some cases, table 2 for example, a direct comparison is made between the various groups of eggs from a single female. An = sign between two columns indicates the embryos being compared are indistinguishable. A > sign indicates the embryos in the left column are more

advanced. The appearance of the first cleavage furrow, Stage 3, is the zero hour for all experiments. The crosses are abbreviated, with the female parent preceding the male parent. Thus, Vt.×Okl. signifies a hybrid derived from a Vermont *pipiens* ♀ and an Oklahoma *pipiens* ♂.

EXPERIMENTAL RESULTS

A. Preliminary crosses between Vermont and Englewood, Florida pipiens

A brief report of these experiments has been published (MOORE 1941), and a résumé will now be given. In the first experiment these embryos were studied:

Englewood, Fla. ♀ × Englewood, Fla. ♂ Vermont ♀ × Vermont ♂
*Englewood, Fla. ♀ × Vermont ♂ *Vermont ♂ × Englewood, Fla. ♂
(in each experiment an * signifies a cross not described previously in this paper). Both types of hybrids were retarded in development, the Englewood, Fla. × Vt. more so than the reciprocal cross. The head of the Englewood, Fla. × Vt. embryos was much reduced in size.

Five additional crosses of Vt. × Englewood, Fla., were made later. In each case the hybrids were extremely abnormal and the mortality was usually 100 percent. The head of these embryos was greatly enlarged, and the circulatory system was defective.

These preliminary experiments indicated a high degree of hybrid inviability in crosses between Vermont and Englewood, Fla., *pipiens*.

B. Vermont and Oklahoma pipiens crosses

In experiment B the following combinations were made:

Vermont ♀ × Vermont ♂
*Vermont ♀ × Oklahoma ♂

Two Vermont females were used, and eggs from both were fertilized with each type of sperm. Data for rate of development at 18.5° in one experiment are given in table 1 (the other gave identical results).

Careful examination in critical stages, such as Stages 13, 14, and 20, revealed no difference in rate between hybrids and controls. There was no evidence of circulatory defects which were characteristic of the Vt. × Englewood, Fla. embryos. There was a slight tendency for the anterior end of the hybrids to be enlarged.

Twenty-four Vt. controls, 48 Vt. × Okl., and 17 Okl. controls were raised to metamorphosis. The three groups of transformed young exhibited pigmentation differences by which they could be distinguished. The average number of spots on the dorsal surface was as follows: Vt. 5.2 ± 0.32; Vt. × Okl. 7.0 ± 0.20; Okla. 9.6 ± 0.47. The spots were larger in the Vt. controls than in the Okl. controls. The hybrids were intermediate in this respect.

This normal development of the Vt. × Okl. embryos was in marked contrast

to the abnormal development of the Vt.×Englewood, Fla. cross. This is astonishing, since it might have been predicted that the Oklahoma and Florida individuals would behave in a similar manner. Thus, both Oklahoma and Florida meadow frogs have been included in the same species (*Rana sphenocephala*) or subspecies (*Rana pipiens sphenocephala*) and delimited from the Vermont

TABLE 1

Stage in development of Vermont controls and Vermont ♀ ×Oklahoma ♂ hybrids. Experiment begun March 30, 1941. Temperature 18.5±0.10°.

AGE IN HOURS	VT.×VT.	VT.×OKL.
0.0	3	3
18.5	9	9
23.0	10E	10E
25.5	10E	10E
30.0	11	11
42.3	12L	12L
50.3	14E	14E
56.5	14L	14L
67.0	17E	17E
74.0	17	17
97.3	18	18
114.3	20E	20E

form (*Rana pipens*, or *Rana brachycephala*, or *Rana pipiens brachycephala*). This distinction is without basis. I regard all meadow frogs as belonging to one species, with no clearly marked subspecies, for the reasons given in MOORE (1944).

The Vermont and Englewood, Florida populations are effectively isolated through the failure of their hybrids to develop. The Vermont and Oklahoma populations are not so isolated.

C. Vermont, Louisiana, and Englewood Florida pipiens crosses

In experiment C the following crosses were made:

Vermont ♀ ×Vermont ♂	Louisiana ♀ ×Louisiana ♂
*Vermont ♀ ×Louisiana ♂	*Louisiana ♀ ×Vermont ♂
Vermont ♀ ×Englewood, Fla. ♂	*Louisiana ♀ ×Englewood, Fla. ♂

The six classes of embryos were kept at 21.9° and at 24.0°. Data for rate of development at 24.0° are given in table 2.

The Vt.×Englewood, Fla. hybrids were similar to those previously described (Experiment A). The first definite retardation in development was noticed at 29.7 hours in the 24.0° experiment. In the controls the neural folds were clearly visible at this time, but in the hybrids they were not very definite. This retardation was observed throughout later development. The typical morphological abnormalities associated with this cross were noticed—namely,

the enlarged yolk plug, wide separation of the neural folds in the anterior end, enlarged heads, and defective gill circulation. These abnormalities did not result in any marked inviability.

The Vt.×La. hybrids exhibited the same type of abnormality observed in the Vt.×Englewood, Fla. cross but to a considerably lesser degree. Retardation in rate of development may have begun in Stage 14 (29.7 hours in the 24.0° experiment), but it was not definite until the controls were in Stage 16 (42 hours in the 21.9° experiment). Both hybrids and controls entered Stage 20 at the same time in the 24.0° experiment. In the 21.9° experiment a slight retardation was noticed in this Stage. Thus, at 80 hours more than half the normals had gill circulation and therefore were in early Stage 20, but fewer than half of the hybrids had corpuscles moving through the gills and so were late Stage 19. This difference represents a retardation of approximately one hour. These hybrids exhibited slightly enlarged heads and somewhat sluggish gill development. These minor abnormalities did not result in death.

TABLE 2

Stage of development of Vermont and Louisiana controls and hybrids of Vermont ♀ × Louisiana ♂, Vermont ♀ × Englewood Florida ♂, Louisiana ♀ × Vermont ♂, and Louisiana ♀ × Englewood Florida ♂. Experiment begun November 5, 1942. Temperature 24.0 ± 0.03°.

AGE IN HOURS	VT.×VT.		VT.×LA.		VT.×FLA.	AGE IN HOURS	LA.×LA.		LA.×VT.		LA.×FLA.
0.0	3		3		3	0.0	3		3		3
19.2	11		—		12	19.2	12M		12M		12M
23.0	12L		12L	>	12L	24.0	14E	=	14E	=	14E
25.3	13	=	13	=	13	25.3	14E	=	14E	=	14E
27.0	13L	=	13L	=	13L	27.0	14M	=	14M	>?	14
29.7	14E	>?	14E	>	14E?	29.7	14L		14L		14L
32.0	14M	>?	14	>	14E	31.0	15	=	15	=	15
42.0	17	>	17	>	17E	32.0	16	>?	16	=	16
49.5	17	>	17	>	17	42.0	18	>	17	=	17
67.0	20E	=	20E	>	17–20	49.5	18	=	18	=	18
						67.0	20	=	20	=	20

The observations on hybrids derived from the Louisiana female are of interest, since this is the first experiment in which an individual from an intermediate locality was crossed with individuals from more northern and from more southern localities. The differences among La. controls, La.×Vt., and La.×Englewood, Fla., are small. There was evidence of a very slight retardation of hybrids at 42 and 46 hours in the 21.9° experiment and at 42 hours in the 24.0° experiment. When the hybrids and controls were entering Stage 20, which is an excellent stage for detecting slight differences in rate, they were developing synchronously. This was true of 21.9° embryos as well as of those developing at 24.0°. The body proportions of the La.×Vt. were identical with those of the controls. The anterior end of the La.×Englewood, Fla. was enlarged to approximately the same degree as was observed in the Vt.×La. embryos.

These experiments confirm the previous observation that Vt.×Englewood, Fla. hybrids are considerably retarded in rate of development and may exhibit constant morphological defects such as enlarged heads, shortened bodies, and defective circulatory systems. They also revealed that development in Vt.×La. embryos is nearly normal. The abnormalities they show are of the same type, but expressed to a considerably lesser degree, as are observed in the Vt.× Englewood, Fla. hybrids. The results with the Louisiana female are of considerable interest. Hybrids derived from these eggs, and sperm of either Vermont or Englewood, Fla. males are so nearly normal that close observation is needed to detect any differences from the controls. Thus, although genetic differences between Vermont and Englewood, Fla. individuals are so great that their hybrids show severe abnormalities, both are able to cross with normal results with Louisiana individuals.

Another experiment was performed in which Vermont and Louisiana eggs were fertilized with Vermont, Louisiana, and Englewood, Fla. sperm. The results were slightly different from the first experiment. The Vt.×Englewood, Fla. embryos had the abnormalities characteristic of this cross developed to a much greater degree than in the previous experiment. When the controls were in Stage 20 not one of these hybrids had a beating heart. Subsequently some developed heart beat and gill circulation, but in the majority no corpuscles were ever observed moving through the gills. The Vt.×La. hybrids were slightly retarded in rate of development, and a few had shortened bodies and slightly defective gill circulation. Development of the La.×Vt. embryos was normal except for a possible slight retardation in rate. The La.×Englewood, Fla. embryos developed at the maternal rate. Neither group of hybrids derived from the Louisiana eggs exhibited noticeable morphological abnormalities.

D. New Jersey, Louisiana, Ocala Florida, and Englewood Florida pipiens crosses

In experiment D the following crosses were made:

Ocala, Fla. ♀ ×Ocala, Fla. ♂ New Jersey ♀ ×New Jersey ♂
*Ocala, Fla. ♀ ×Englewood, Fla. ♂ *New Jersey ♀ ×Louisiana ♂
*Ocala, Fla. ♀ ×Louisiana ♂
*Ocala, Fla. ♀ ×New Jersey ♂

The females are from localities not previously used in cross fertilization experiments. Data for rate of development are given in table 3.

The rate of development of the controls and hybrids derived from the Ocala, Fla. eggs was identical. At 46 hours all groups were in middle Stage 14. During this period morphological changes are rapid, so any slight difference in degree of development could be detected. However, no differences were apparent At 97 hours half of the Ocala, Fla.×Englewood, Fla. embryos were in Stage 20. A few, but not half, of the Ocala controls and the Ocala, Fla.×La. hybrids were in Stage 20 at this time. The controls reached Stage 20 at 98.5 hours. The Ocala, Fla.×La. and Ocala, Fla.×N.J. hybrids reached this stage at 102.3

hours. When the four groups were examined at 118.3 hours, the controls were still in Stage 20. On the basis of gill development it appeared that the Ocala, Fla.×Englewood, Fla. hybrids were slightly more advanced. The Ocala, Fla.× La. embryos may have been slightly retarded. The Ocala, Fla.×N.J. were slightly, but definitely, retarded. No differences in viability could be detected among the four groups of embryos.

TABLE 3

Stage of development of Ocala, Florida and New Jersey controls and hybrids of Ocala, Florida ♀×Englewood, Florida ♂, Ocala, Florida ♀×Louisiana ♂, Ocala, Florida ♀×New Jersey ♂, and New Jersey ♀×Louisiana ♂. Temperature in all cases was 19.1±.03°. Experiment begun November 26, 1942.

AGE IN HOURS	OCALA ×OCALA		OCALA ×ENGLE.		OCALA ×LA.		OCALA ×N.J.	AGE IN HOURS	N.J.×N.J.		N.J.♂LA.
0.0	3		3		3		3	0	3		3
22.0	10L	=	10L	=	10L	=	10L	22.0	10M	=	10M
46.0	14M	=	14M	=	14M	=	14M	46.0	14M	=	14M
50.3	16E	=	16E	=	16E	=	16E	50.5	14L	=	14L
55.8	16L	=	16L	=	16L	=	16L	68.7	17	=	17
68.3	17L	=	17L	=	17L	=	17L	95.3	19	?<	19
93.0	19		19		19		19	98.8	19		19
97.0	19L		20E		19L		19L	100.0	20	=	20
98.5	20E		—		19L		19L	118.3	20	=	20
102.3	—		—		20E		20E				
118.3	20	?<	20	>?	20	>	20				

Careful examination of New Jersey controls and N.J.×La. hybrids in stages during which slight differences in rate could have been detected showed the two groups to be identical. There was no reduction of viability in the hybrids.

This experiment indicated that Ocala, Florida *pipiens* eggs can be fertilized with sperm of Englewood Florida, Louisiana, or New Jersey males, and the resulting hybrids develop in an almost, or completely normal manner. Eggs of New Jersey females develop normally when fertilized with sperm of Louisiana males.

E. Vermont, New Jersey, Ocala Florida, and Englewood Florida pipiens crosses

In experiment E the following crosses were made:

Vermont ♀×Vermont ♂	Ocala, Fla. ♀×Ocala, Fla. ♂
Vermont ♀×New Jersey ♂	Ocala, Fla. ♀×Englewood, Fla. ♂
*Vermont ♀×Ocala, Fla. ♂	Ocala, Fla. ♀×New Jersey ♂
Vermont ♀×Englewood, Fla. ♂	*Ocala, Fla. ♀×Vermont ♂
	New Jersey ♀×New Jersey ♂
	*New Jersey ♀×Ocala, Fla. ♂

These eggs were raised at 19.3° and 21.8°.

Data for rate of development at 21.8° in the Ocala, Fla. controls and hybrids are given in table 4. No differences in either rate or normality of development could be detected between the Ocala, Fla. controls and the Ocala, Fla.×Englewood, Fla. hybrids. This was true not only for the 21.8° eggs but also for those developing at 19.3°.

TABLE 4

Stage of development of Ocala, Florida and Vermont controls and hybrids of Ocala, Florida ♀×Englewood, Florida ♂, Ocala, Florida ♀×New Jersey ♂, Ocala, Florida ♀×Vermont ♂, Vermont ♀×New Jersey ♂, Vermont ♀×Ocala, Florida ♂ and Vermont ♀×Engelwood, Florida ♂. Temperature 21.8±0.02°C. Experiment begun December 10, 1942.

AGE IN HOURS	OCALA ×OCALA		OCALA ×ENGLE.		OCALA ×N.J.		OCALA ×VT.	VT.×VT.		VT.×N.J.		VT. ×OCALA		VT.× ENGLE.
0.0	3		3		3		3	3		3		3		3
21.5	12M	=	12M	=	12M	>	12E	12E	=	12E	=	12E	=	12E
24.5	13	=	13	>	13E	>	12L	12	=	12	=	12	=	12
28.3	13L		13L		—	>	13L	12L		—		—		—
31.5	14E		14E		14E		14E	13	=	13	=	13	=	13
44.5	17E	=	17E	>	16L	>	16	16E	=	16E	>?	16E	>	16
47.0	17		17		17	>	17	16L		—		—		16
51.5	17M		17M		17M	>?	17M	17E		17E	>	16L		17E
55.3	18		18		18	>	18	17		17		17		17
70.0	19L	=	19L	>	19	>	19	18		18	>	18		18
73.5	20E	=	20E	>	19	>	19	19		19	>	19	>	18
78.5	—		—		20E	>	20E	20E	>?	20E	>	19	=	19

The Ocala, Fla.×N.J. hybrids were slightly retarded in rate. In addition the type of morphological defect characteristic of hybrids formed from southern eggs and northern sperm was noticed. In Stage 14 the neural folds were not elevated as much as in the controls, and in the future brain region they were closer together. Some of these developed into tadpoles with deficient head structures, the most noticeable of which were the absence of a mouth, fusion of olfactory pits, and poor development of the brain. When these embryos were classified in the thirteenth day, it was found that 7 percent had no mouth. In the remaining 93 percent the mouth was nearly or completely normal.

The Ocala, Fla.×Vt. hybrids exhibited the same type of defects as seen in the Ocala, Fla.×N.J. hybrids but to a greater degree. There was considerable variation in rate of development, with many showing extreme retardation. The morphological abnormalities consisted of a reduction in head structures. In the more extreme cases the head was very narrow, the eyes close together at the front of the embryo, and the suckers and olfactory pits were fused. On the thirteenth day 55 percent lacked an open mouth.

The Vermont controls and hybrids derived from Vermont eggs were also kept at 19.3° and at 21.8°. Data for the latter temperature are given in table 4. The Vt.×N.J. hybrids developed normally, except for a slight retardation in rate noticed during Stage 20 in the 21.8°, and in Stages 14, 15, 16, and 20 in

the 19.3° experiment. Retardation in rate in the Vt.×Ocala, Fla., and Vt. ×Englewood, Fla. hybrids was first noticed in Stage 14 in the 19.3° experiment, and in Stage 16 in the 21.8° experiment. The hybrids derived from the Englewood male were more retarded than those from the Ocala male. Circulatory system disturbances were present. These were more serious in the Vt.× Englewood, Fla. than in the Vt.×Ocala, Fla. hybrids.

TABLE 5

Stage of development of New Jersey controls and New Jersey ♀×Ocala, Florida ♂ hybrids. Temperature 21.8±0.02°C. Experiment begun December 10, 1942.

AGE IN HOURS	N.J.×N.J.		N.J.×OCALA
0.0	3		3
12.0	9		9
14.8	9L	=	9L
16.5	10E	=	10E
22.0	12E	=	12E
34.8	14E	>	14E
37.0	14M	>	14M
41.5	16E	>	16E
45.5	16		16
60.5	18	>	18
82.0	20	=	20

Eggs of the New Jersey female were hybridized with the Ocala, Fla. male. A slight retardation in rate was noticed in Stage 14 (table 5) but not in Stage 20. On the ninth day the hybrids were definitely retarded. Gill circulation was sluggish in Stage 20, but the embryos recovered later.

Four new combinations were made in this experiment: Vt.×Ocala, Fla.; Vt.×N.J.; N.J.×Ocala, Fla.; Ocala, Fla.×Vt. The Ocala, Fla.×Vt. hybrids had reduced heads and suffered a retardation in rate similar to that observed in Englewood, Fla.×Vt. embryos (experiment A). The Ocala, Fla.×N.J. hybrids were more nearly normal, suffering only slight retardation in rate and morphological defects. In the reciprocal cross, N.J.×Ocala, Fla., the retardation and morphological defects were also slight. The Vt.×N.J. embryos were nearly completely normal.

PORTER (1941) was the first to cross *pipiens* from Vermont and New Jersey or Pennsylvania (his material was collected along the Schuylkill and Delaware Rivers in the Philadelphia region; my "New Jersey" material is from the same source). He reports a very slight head enlargement in the Vt.×Penn. hybrids and a slight head reduction in the Penn.×Vt. hybrids.

F. Vermont, New Jersey, Ocala Florida, and Englewood Florida, pipiens crosses

In experiment F the following crosses were made:

Vermont ♀ × Vermont ♂
Vermont ♀ × New Jersey ♂
Vermont ♀ × Ocala, Fla. ♂
Vermont ♀ × Englewood, Fla. ♂
New Jersey ♀ × New Jersey ♂
*New Jersey ♀ × Vermont ♂
New Jersey ♀ × Ocala, Fla. ♂
*New Jersey ♀ × Englewood, Fla. ♂
Ocala, Fla. ♀ × Vermont ♂
Ocala, Fla. ♀ × New Jersey ♂
Ocala, Fla. ♀ × Ocala, Fla. ♂
Ocala, Fla. ♀ × Englewood, Fla. ♂

The embryos were raised at 19.2°. This experiment was important, since nearly every combination of east-coast individuals was available at the same time for comparison.

Data for development of the Vermont controls and hybrids derived from this egg are given in table 6. The Vt.×N.J. hybrids were entirely normal in rate and morphology. The Vt.×Ocala, Fla. hybrids were slightly retarded in Stage 14 and subsequently. The Vt.×Englewood, Fla. embryos were even more retarded. At 108 hours both the controls and Vt.×N.J. were in Stage 20. On the basis of gill development it was concluded that they had been in this stage for ten hours. The Vt.×Ocala, Fla. embryos were obviously retarded. They were in early Stage 20. The anterior end was noticeably enlarged. The Vt.×Englewood, Fla. hybrids were greatly retarded and highly variable at this time. In some the heart had not begun to beat, and only a very few had gill circulation. Most of these recovered from the early circulatory defects, and by 228.5 hours they were as nearly normal as the controls.

TABLE 6

Stage of development of Vermont and New Jersey controls and hybrids of Vermont ♀ × New Jersey ♂, Vermont ♀ × Ocala, Florida ♂, and Vermont ♀ × Englewood, Florida ♂, and New Jersey ♀ × Vermont ♂, New Jersey ♀ × Ocala, Florida ♂, and New Jersey ♀ × Englewood, Florida ♂. Temperature 19.2 ± 0.02°. Experiment begun December 23, 1942.

AGE IN HOURS	VT.×VT.		VT.×N.J.		VT. ×OCALA		VT. ENGLE.	N.J.×N.J.		N.J.×VT.		N.J. ×OCALA		N.J. ×ENGLE.
0.0	3		3		3		3	3		3		3		3
36.5	12L		12L		12L		12L	13E	=	13E	=	13E	=	13E
46.0	14E	=	14E	>	14E	>	14E	14M	=	14M	>	14E	>?	14E
61.3	16L	=	16L	>	16	=	16	17E	=	17E	>	17E		17E
94.5	19E	=	19E	>	18	=	18	19	=	19	>	19	>	18
108.0	20	=	20	>	20E	>	18–19	20	=	20	=	20	=	20

Data for the hybrids derived from the New Jersey eggs are also given in table 6. The N.J.×Vt. hybrids were normal in rate and body proportions. The N.J.×Ocala, Fla. and the N.J.×Englewood, Fla. embryos were slightly retarded in early stages, but by the time they reached 108 hours they were identical with the controls. In a few individuals slight circulatory defects were noticed. The anterior end of the N.J.×Ocala, Fla. embryos was slightly en-

larged. This abnormality was accentuated in the N.J.×Englewood, Fla. hybrids.

Data for the Ocala, Fla. normals and hybrids are given in table 7. The Ocala, Fla.×Englewood, Fla. hybrids were entirely normal in rate and body proportions. When these eggs were fertilized with sperm of northern individuals, the hybrids were retarded and had reduced heads. At 108.3 hours the Ocala, Fla.×N.J. were not so well developed as the controls and the head was narrow. The Ocala, Fla.×Vt. at this same time were even more retarded and their heads were very narrow. The suckers were fused in nearly every embryo and a few lacked a stomodaeum.

TABLE 7

Stage of development of Ocala, Florida, controls and hybrids of Ocala, Florida ♀×Englewood, Florida ♂, Ocala, Florida ♀×New Jersey ♂, and Ocala, Florida ♀×Vermont ♂. Temperature 19.2±0.02°. Experiment begun December 23, 1942.

AGE IN HOURS	OCALA×OCALA		OCALA×ENGLE.		OCALA×N.J.		OCALA×VT.
0.0	3		3		3		3
36.7	13L	=	13L		13E		13L
46.0	14M	=	14M	=	14M	=	14M
61.5	17E	=	17E	>	16		16
95.3	19	=	19	>	19	>	18
108.3	20	=	20	>	20E	>	20E

Most of the data so far presented have involved crosses of eastern American frogs. These populations can be arranged in a series that coincides with their north-south distribution and their behavior in hybridization: (1) Vermont, (2) New Jersey, (3) Louisiana, (4) Ocala, Florida, (5) Englewood, Florida. Adjacent members of the series produce normal or nearly normal embryos. In combinations between progressively more distant members of the series, morphological defects and retardation of development become more pronounced. In crosses between the end members of the series these defects become so pronounced that in some experiments there is complete hybrid inviability. The types of morphological abnormality follow definite patterns: (a) hybrids derived from northern eggs and southern sperm tend to have enlarged heads and circulatory system defects; (b) hybrids derived from southern eggs and northern sperm tend to have reduced heads with fused suckers, fused olfactory pits, and frequently with the mouth absent.

G. Texas, Wisconsin, and Vermont pipiens crosses

In experiment G the following crosses were made:

Texas ♀×Texas ♂	Wisconsin 1 ♀×Wisconsin ♂
*Texas ♀×Vermont ♂	*Wisconsin 1 ♀×Texas ♂
*Texas ♀×Wisconsin ♂	Wisconsin 2 ♀×Wisconsin ♂
	*Wisconsin 2 ♀×Texas ♂

The embryos were raised at 19.2° and at 22.6°. Wisconsin *pipiens* resemble the Vermont *pipiens* closely in both adult and embryological characters. No indication of hybrid abnormality has been observed in any of the numerous crosses between individuals from these two localities (no data will be given for this cross). In crosses between Texas and northern individuals the hybrids exhibited the same type of abnormality previously observed in the Vermont and Florida crosses but expressed to a greater degree.

Data for developmental rate at 19.2° are given in table 8. The development of the Texas×Wis. and Texas×Vt. embryos was essentially the same. At 42.7 hours hybrids and controls were in Stage 14. The neural folds of the hybrids were very close together in the anterior region. This foreshadows the small head that will appear in the later embryo. No retardation was noticed

TABLE 8

Stage of development of Texas and Wisconsin controls and hybrids of Texas ♀ × Vermont ♂, Texas ♀ × Wisconsin ♂, and Wisconsin ♀ × Texas ♂. Temperature 19.2 ± 0.02°. Experiment begun October 11, 1944.

AGE IN HOURS	TEXAS ×TEXAS		TEXAS ×VT.		TEXAS ×WIS.	AGE IN HOURS	WIS. ×WIS.		WIS. ×TEXAS
0.0	3		3		3	0.0	3		3
16.0	9		9		9	15.8	9		9
18.5	9L	=	9L	=	9L	18.5	9	>?	9
20.0	10E	=	10E	=	10E	20.0	10E	>	10E
23.0	10M	=	10M	=	10M	23.0	10	=	10
27.5	11L	=	11L	=	11L	27.5	11L	=	11L
40.5	13	>?	13	>?	13	40.5	13	>	13
42.7	14E	=	14E	=	14E	42.5	14E	>	13
44.7	14E	=	14E	=	14E	45.0	14E	>	13
51.0	14M	<	14L	=	14L	51.0	16E	>	14E
65.0	17E	<	17	=	17	65.0	17	>	16
71.3	17		17		17	71.3	18	>	17
90.0	18		18		18	90.0	19E	>	18
99.5	19		19		19	99.5	20E	>	18
105.3	20E	>	19	=	19	143.0	21	>	20
118.0	20	>	20E	=	20E				
143.0	21	>	20	=	20				

at this time; in fact the neural folds of the hybrids closed slightly earlier than the normals. At 71.3 hours it was noticed that most of the hybrids of both groups lacked olfactory pits and a stomodaeum. Hatching was retarded, and by 105.3 hours they were well behind the controls in morphological development. These hybrids were very abnormal. The suckers were greatly reduced in size and were usually fused. The ventral portion of the head projected as a trunk-like appendage. The olfactory pits were either very close together or completely fused. The mouth was small or absent. These abnormalities occurred at 19.2° and 22.6°. On the fifteenth day 36 Texas×Wis. hybrids were examined for the degree of head development. The results were as follows: 19

lacked a mouth, horny jaws, teeth, and a spiracle; 13 others lacked an open mouth and spiracle but possessed some mouth structures such as horny jaws, papillae, and teeth; four embryos (11 percent) had an open mouth and spiracle through which water was moving. On the sixteenth day 36 Texas×Vt. embryos were examined. The results were as follows: 23 lacked a spiracle, mouth, and mouth parts such as horny jaws, teeth, and papillae; 12 possessed some mouth structures but no spiracle; 1 embryo possessed both a mouth and spiracle.

The eggs of two Wisconsin females were fertilized with Texas sperm. Embryos were raised at both 19.2° and at 22.6°. Hybrids from both of the females behaved in a similar manner. Rate data for one group from 19.2° are given in table 8. Retardation was definite in the hybrids by Stage 14 and marked in subsequent stages. The circulatory disturbances previously noticed in the Vt.×Englewood, Fla. hybrids were present. It was not until 143 hours that the Wis.×Texas embryos had good gill circulation. These embryos were further characterized by tremendous head development with large mouths, olfactory pits, and suckers. At 22.6° the same retardation and defects were noticed. On the sixth day, when the controls were in Stage 23, 55 percent of the hybrids did not have gill circulation. In the remaining 45 percent gill circulation varied from sluggish to normal. All of these 22.6° hybrids were dead by the ninth day. At 19.2° many survived even though they were abnormal.

This experiment indicated that the Texas *pipiens*, when crossed with Vermont or Wisconsin *pipiens*, show the same type of hybrid defect observed when the Englewood, Florida individuals are crossed with the same northern forms.

H. Texas and Wisconsin pipiens crosses

A second experiment was performed in which Texas and Wisconsin controls and their reciprocal hybrids were compared. The results were similar to, but more extreme than, those obtained in experiment G. In the Wis.×Texas group only 4 percent had gill circulation and all died, or were nearly dead, by the tenth day. These had the greatly exaggerated head structures noticed previously. The Texas×Wis. hybrids classified on the eleventh day had very reduced heads. The results were as follows: 88 percent lacked mouth structures such as jaws, teeth, and papillae; 95 percent were without an open mouth; 100 percent lacked a spiracle; 73 percent did not have an anus; 55 percent had eyes absent or so reduced that they could not be seen externally. Not one survived.

I. Texas and Vermont pipiens crosses

In this experiment the eggs of a Vermont *pipiens* were fertilized with sperm of a Vermont male and with sperm of a Texas male. The results were essentially similar to those obtained in the Wis.×Texas cross of Experiment G. Retardation in rate of development was noticed when the controls were in Stage 14 and this increased in later stages. The anterior end of the hybrids was considerably enlarged and the circulatory system was defective. These are the types

of abnormalities that have occurred repeatedly in crosses between northern females and southern males.

This experiment was performed primarily to study the effect of temperature on the expression of the hybrid abnormalities.

At 27.5° the controls were in Stage 22 at 93.7 hours. An examination of the embryos for gill circulation and heart beat at this time revealed the following: in 47 percent the heart was not beating; in 10 percent the heart was beating but there was no gill circulation; in 43 percent gill circulation was sluggish. These embryos were very abnormal and none survived.

At 19.0° the hybrids were more nearly normal. The head and head structures were greatly enlarged. The circulatory system was more nearly normal than at 27.5°, and when the controls were in late Stage 21, 91 percent of the hybrids had fairly normal gill circulation. Embryos of this group overcame these early circulatory defects and at 215 hours were developing normally.

A third group of eggs was kept at 14.3°. When the controls were in Stage 22 one embryo from the total of 21 had nearly normal gill circulation. Another embryo had poor gill circulation. The remaining 19 (90 percent) did not have any gill circulation. Not one was normal enough to survive.

The hybrids raised at 9.8° disintegrated by the time the controls were in Stage 18.

This experiment, in addition to revealing the expected type of abnormalities, indicated that the degree of abnormality is greatly influenced by temperature. At an intermediate temperature of 19.0° development is much better than at any of the higher or lower temperatures employed.

J. Vermont, New Jersey, Ocala Florida, and Texas pipiens crosses

In Experiment J the following crosses were made:

New Jersey ♀ × New Jersey ♂	Texas ♀ × Texas ♂
New Jersey ♀ × Vermont ♂	*Texas ♀ × New Jersey ♂
*New Jersey ♀ × Texas ♂	Texas ♀ × Vermont ♂
New Jersey ♀ × Ocala, Fla. ♂	*Texas ♀ × Ocala, Fla. ♂

The crosses involving the Ocala, Fla. male were made five hours later than the other combinations, and these two groups of hybrids were raised at 19.0°. The controls and other hybrids were raised at 19.0° and at 27.3°.

Data for the rate of development in the six groups of eggs fertilized with sperm of New Jersey, Vermont, and Texas males are given in table 9. No difference in rate of development was observed between New Jersey controls and N.J. × Vt. hybrids. This was a confirmation of several previous experiments. The N.J. × Texas hybrids were retarded at 43.5 hours when the controls were in Stage 14. The distance between the neural folds in the head region was abnormally great. In subsequent stages these embryos were characterized by large heads with overdeveloped suckers and olfactory pits. The onset of heart beat and gill circulation was much delayed. These embryos were misshapen and

by 235 hours nearly all were dead. Although the head was definitely enlarged, it was probably not as large as in the Vt.×Texas cross. This point could not be definitely established, since Vt.×Texas hybrids of the same age were not available for comparison.

TABLE 9

Stage of development of New Jersey and Texas controls and hybrids of New Jersey ♀×Vermont ♂, New Jersey ♀×Texas ♂, Texas ♀×New Jersey ♂, and Texas ♀×Vermont ♂. Experiment begun October 13, 1945. Temperature 19.0±0.06°.

AGE IN HOURS	N.J.×N.J.	N.J×VT.	N.J. ×TEXAS	AGE IN HOURS	TEXAS ×TEXAS	TEXAS ×N.J.	TEXAS ×VT.
0.0	3	3	3	0.0	3	3	3
20.0	10E =	10E =	10E	20.0	10E =	10E >?	10E
24.3	11E ?<	11 ?<	11L	24.3	10M =	10M =	10M
25.5	11	11 <	12E	29.5	12E =	12E =	12E
29.5	12	12	12	43.5	13L =	13L =	13L
43.5	14E =	14E >	13	49.0	14E <	14M	14M
49.0	14L =	14L >	14E	54.0	14M <	14L =	14L
54.0	16E =	16E >	14L	67.5	17 ?<	17 ?<	17
67.3	17M =	17M >	17E	94.5	18 =	18 =	18
95.0	19	19	—	104.5	19 =	19 =	19
100.7	20E	20E	—	113.0	20E =	20E >	19?
104.5	20	20	18	122.0	—	—	20E
116.3	20M	—	18–20E	146.5	21 >?	20	20
146.8	21	—	20	163.0	21L	21L	21

The Texas×Vt. hybrids had previously been studied (Experiment G), but the Texas×N.J. combination was new. This experiment thus afforded an opportunity to compare the degree of abnormality in Texas×N.J. with the Texas×Vt. hybrids. Both groups of hybrids were slightly ahead of the controls during the neurula stages, but in Stages 18 and 19 the three groups were identical (slight differences in rate would be masked during these stages). The Texas controls and Texas×N.J. embryos entered Stage 20 simultaneously. The Texas×Vt. hybrids were considerably retarded at this time (113 hours). Heart beat could not be detected, although on the basis of gill development the embryos were probably in Stage 19. Nine hours later a few were in Stage 20. As far as morphological defects were concerned the two groups of hybrids were similar in having reduced heads. However, the degree of abnormality was much more extreme in the Texas×Vt. hybrids than in the Texas×N.J. embryos. Many of the former were entirely without a mouth.

The N.J.×Ocala, Fla. hybrids had slightly enlarged heads, but gill circulation appeared normal. No rate data will be given for this cross, because the embryos were not developing synchronously with the controls.

The Texas×Ocala, Fla. cross was likewise not made until five hours after the other crosses (no rate data will be given). It was not possible therefore to make an exact comparison, but as well as could be told these hybrids were entirely normal in rate and in body proportions. This finding was of great inter-

est. It revealed that in spite of the pronounced morphological and embryological differences that separate the Florida and Texas forms, there was no incompatability of a type leading to hybrid inviability. Their similar behavior in hybridization with northern forms is probably due to a similar genetic background.

These experiments also revealed that the Texas×N.J. hybrids were more nearly normal than the Texas×Vt. Here again we notice an increase in hybrid abnormality with an increase of latitude distance between the parent populations.

K. Vermont, New Jersey, Ocala Florida, and Texas pipiens crosses

In experiment K the following crosses were made:

Vermont ♀ × Vermont ♂	Ocala, Fla. ♀ × Ocala, Fla. ♂
Vermont ♀ × New Jersey ♂	*Ocala, Fla. ♀ × Texas ♂
Vermont ♀ × Ocala, Fla. ♂	Ocala, Fla. ♀ × New Jersey ♂
Vermont ♀ × Texas ♂	Ocala, Fla. ♀ × Vermont ♂

Texas ♀ × Texas ♂
Texas ♀ × Ocala, Fla. ♂
Texas ♀ × New Jersey ♂
Texas ♀ × Vermont ♂

The eggs of one Vermont, one Texas, and two Ocala, Fla. females were fertilized with sperm of the four types of males. The normals and hybrids derived from the Vermont, Texas, and one of the Ocala, Fla. females were raised at 19.0°. The normals and hybrids derived from the second Ocala, Fla. female were raised at 23.2°. Most of these combinations have been described previously, so extensive rate data will not be given. This experiment was especially valuable, because it offered an opportunity to make direct comparison between many different combinations. It might be emphasized again that the gradient of increasing hybrid abnormality is best indicated by close comparison of different hybrids derived from the eggs of the same female.

The hybrids derived from the Vermont eggs showed a graded series of abnormalities. At 49 hours both Vermont controls and Vt.×N.J. hybrids were middle Stage 14. The Vt.×Ocala, Fla. were early 14, and the Vt.×Texas a still earlier Stage 14. By 102 hours the Vermont controls and Vt.×N.J. hybrids were early Stage 20. The Vt.×Ocala, Fla. were recorded as late Stage 19 on the basis of gill development. The Vt.×Texas embryos were much retarded, being in Stage 18. Morphological abnormalities were of the expected type. The Vt.×N.J. hybrids had slightly enlarged heads. The heads of the Vt.× Ocala, Fla. embryos were greatly enlarged, and this defect was even more exaggerated in the Vt.×Texas hybrids. Circulatory system defects were not extreme. As usual the Vt.×N.J. embryos were entirely normal in this respect. The Vt.×Ocala, Fla. hybrids exhibited minor defects at the time heart beat and gill circulation were beginning, but by 188.7 hours they had recovered. At 188.7 hours 60 percent of the Vt.×Texas embryos had nearly normal gill circulation, in 20 percent it was poor, and in 20 percent it was absent.

The controls and hybrids from one of the Ocala, Fla. females were raised at 19.0°. During the neural fold stages these Ocala, Fla. controls and Ocala, Fla.×N.J. and Ocala, Fla.×Vt. hybrids were identical in rate. The Ocala, Fla.×Texas hybrids were slightly retarded. At 98 hours the controls were early Stage 20, the Ocala, Fla.×Texas late Stage 19, the Ocala, Fla.×N.J. an earlier Stage 19, and the Ocala, Fla.×Vt. Stage 18. The morphological abnormalities were as follows: the Ocala, Fla.×N.J. hybrids had slightly reduced heads; the Ocala, Fla.×Vt. had the anterior end more reduced and some fusion of head structures; the Ocala, Fla.×Texas had the anterior end slightly, but definitely, *enlarged*. Hybrids from a second Ocala, Fla. female were raised at 23.2°. The results of the two experiments were essentially in agreement. The only difference observed was that the retardation of Ocala, Fla.×Texas hybrids in neural fold stages was not so definite.

None of the crosses with the Texas female were new, but this experiment provided the first opportunity to compare development of Texas×Ocala, Fla. hybrids with controls of the same age. The Texas×Vt. embryos were more retarded than the Texas×N.J. The Texas×Ocala, Fla. hybrids were slightly accelerated in development. Thus, at 113.3 hours the degree of development was as follows: Texas controls late Stage 19; Texas×Ocala, Fla. early Stage 20; Texas×N.J. early Stage 19; Texas×Vt. Stage 18. Body proportions in Texas×Ocala, Fla. embryos seemed normal. The anterior end of the Texas×N.J. embryos was considerably reduced, and in the Texas×Vt. it was even more reduced. In the later group many lacked a mouth.

This experiment is of special interest, since hybrids derived from both Ocala, Fla. and Texas individuals were available for direct comparison. The results indicated t hat both the Ocala, Florida and Texas individuals gave the same type of abnormalities when hybridized with northern forms. However, the defects were more extreme when the Texas individuals were used. The Texas frogs were behaving in a "more southern" manner than those from Ocala, Florida. The Ocala, Fla.×Texas cross was interesting in this respect, since it resulted in hybrids with slightly enlarged heads. This type of defect is characteristic in crosses between northern females and southern males.

The developmental rate of hybrids of Texas and Ocala, Fla. *pipiens* was an interesting exception to the results of the other crosses. The Ocala, Fla.× Texas were retarded in rate, and the Texas×Ocala, Fla. were accelerated. This is the only cross in which the reciprocal hybrids differed in this manner. In all other combinations the hybrids either develop at the same rate as the controls, or they are retarded. The retardation in these many cases is interpreted as showing incompatability of genes influencing development. In the exceptional hybrids, Ocala, Fla.×Texas, it is thought that the "retardation" is actually an intermediate rate in a hybrid derived from the egg of a rapidly developing form and the sperm of a slowly developing form. In the reciprocal cross, Texas×Ocala, Fla., the combination is a "slow" egg and a "fast" sperm. The acceleration noticed in this case is likewise a rate intermediate between that of the parental strains. This is the only cross in which this complication arises. The rate of development in all populations, except Texas, may be very

different at some temperatures, but at 19° they are essentially identical. Retardation in these cases means that the hybrids are developing more slowly than either of the parent strains.

SUMMARY OF CROSSES

The results of 26 crosses between *Rana pipiens* from different localities in the United States are given in table 10. The localities are arranged in a manner that reflects the results of the hybridization experiments. The diagonal extending from the upper left to the lower right corner is the line of normal development. As the distance from this line increases, the abnormalities of development become progressively more pronounced. The cross combinations to the right of this line exhibit increasing head enlargement with associated circulatory system defects, and those to the left show increasing head reduction. The latitude of the populations is given under the male localities. The letters below the descriptions of the hybrids refer to the experiments in which the cross is described.

Some slight variation was encountered on the different occasions when the same type of cross was made. An example can be given for the Vt.×N.J. hybrids which were studied in experiments E, F, and K. In experiment E the hybrids were normal in body proportions but showed a slight retardation in rate of development. In experiment F the hybrids were normal, and in experiment K the rate was normal but the hybrids exhibited slightly enlarged heads.

DISCUSSION

From an evolutionary point of view[3] the principal interest of these observations lies in the gradual manner in which genetic isolation becomes established with increasing geographical distance. The hybrids from a cross between Vermont and south Florida, or between Wisconsin and Texas *pipiens* possess such marked abnormalities that few would survive under natural conditions. In no case, however, were isolating mechanisms of this type found in crosses between individuals of relatively less distant localities. The Vermont×New Jersey, New Jersey×Louisiana, Louisiana×central Florida, and central Florida×southern Florida hybrids were normal. The transfer of some genes from extreme northern to extreme southern populations by successive crossings of individuals from adjacent localities is therefore a possibility under natural conditions.

A similar increase of genetic isolation with increased distance between populations has been noticed in a few cases. GORDON and SMITH (1938) have studied the formation of melanotic neoplasms in crosses among four very closely related species of the genus Platypoecilus. These forms, *P. couchianus*, *P. xiphidium*, *P. variatus*, and *P. maculatus* replace one another from north to

[3] The abnormalities resulting from the crosses are of considerable interest from an embryologic point of view. This aspect of the problem is quite distinct from the evolutionary implications. It will not be considered in this paper.

TABLE 10

Summary of crosses between different populations of Rana pipiens. See text for explanation.

FEMALES	MALES: VERMONT 45° N.	WISCONSIN 44° N.	NEW JERSEY 40° N.	OKLAHOMA 36° N.	LOUISIANA 30° N.	OCALA, FLORIDA 29° N.	ENGLEWOOD, FLORIDA 27° N.	TEXAS 32° N.
VERMONT	Controls	Rate: normal Head: normal G	Rate: normal or very slight retardation Head: normal or very slight enlargement E F K	Rate: normal Head: slight enlargement B	Rate: very slight retardation Head: moderate enlargement C	Rate: moderate retardation Head: considerable enlargement E F K	Rate: marked retardation Head: considerable enlargement A C E F	Rate: marked retardation Head: extreme enlargement I K
WISCONSIN	Rate: normal Head: normal G	Controls						Rate: marked retardation Head: extreme enlargement G H
NEW JERSEY	Rate: normal Head: normal F J		Controls		Rate: normal D	Rate: very slight retardation Head: very slight enlargement E F J	Rate: slight retardation Head: moderate largement F	Rate: marked retardation Head: considerable enlargement J
LOUISIANA	Rate: very slight retardation Head: normal C				Controls		Rate: normal Head: moderate enlargement C	
OCALA, FLORIDA	Rate: marked retardation Head: considerable reduction E F K		Rate: slight retardation Head: slight reduction D E F K		Rate: normal D	Controls	Rate: normal Head: normal D E F	Rate: slight retardation Head: slight enlargement K
ENGLEWOOD, FLORIDA	Rate: marked retardation Head: considerable reduction A						Controls	
TEXAS	Rate: marked retardation Head: extreme reduction G J K	Rate: marked retardation Head: extreme reduction G H	Rate: slight retardation Head: moderate reduction J K			Rate: slight acceleration Head: normal J K		Controls

south in the sequence indicated. Melanosis is severe in hybrids between *couchianus* and *maculatus*, less between *xiphidium* and *maculatus*, and least between *variatus* and *maculatus*. MAINLAND (1942) has observed an increase of genetic isolation with increasing distances between the parent populations in crosses of *Drosophila subfunebris* with different geographical races of *Drosophila macrospina*.

The usual explanation for hybrid inviability in interspecific and intraspecific crosses is that failure is due to the action of complementary lethal genes (DOBZHANSKY 1941, page 282). The first well analyzed case of this was presented by HOLLINGSHEAD (1930). If *Crepis tectorum*, homozygous for the *l* gene, is crossed with *Crepis capillaris*, the hybrids die. The *l* gene has no detectable effect in *Crepis tectorum*, yet in the presence of the *capillaris* genome it acts as a lethal. HUTCHINSON (1931) and SILOW (1941) have described a similar case in Gossypium. *Gossypium aboreum* var. *soudanensis*, which is restricted to Anglo-Egyptian Sudan, is characterized by a gene Cp_a. Some other varieties possess a gene Cp_b. Neither gene has a detectable effect alone, but if both are present in a hybrid, the result is a semi-lethal or lethal effect. CROW (1942) and PATTERSON and GRIFFEN (1944) have described similar cases in Drosophila.

The graded results obtained in crosses of a northern *pipiens* with progressively more southern individuals of the same species indicate that a single pair of complementary lethal genes cannot offer a satisfactory explanation. Instead we must think in terms of "complementary lethal genomes" or perhaps of a series of alleles of a type described in Triticum (SEARS 1944).

The development of hybrid inviability solely for its "usefulness" as an isolating mechanism cannot be due to natural selection in this case. This is apparent, since the *pipiens* populations which are isolated from one another by hybrid inviability are not sympatric. Instead hybrid inviability appears to be developing incidental to the process of divergence. A possible explanation, similar to the views of MULLER (1942) on this subject, will now be given.

The local populations used in these experiments occur in regions with great differences in environmental conditions. In these populations mutations are constantly occurring. A few of these mutations, or combinations of them, may better adapt the individuals to the peculiarities of their local environment. These advantageous genes, and gene combinations, will increase in frequency as a result of natural selection. With the passage of time, genetic differences between populations in contrasting environments may become considerable. The individuals of any locality will possess a delicately adjusted genome of considerable survival value for a definite type of environment. By and large the magnitude of the genetic differences between populations will be proportional to the differences between their environments. If cross fertilizations are made between individuals with very different genetic constitutions, the conflicting modes of action of the maternal and the paternal genes in the hybrid may result in abnormal development.

The north-south gradient of hybrid inviability observed in the experiments suggests that temperature is the principal selecting agent. The same type of

gradient is noticed along the east coast and in the central region. Furthermore, the warm, *moist* environment of Florida and the warm, *dry* environment of western Texas have produced populations that behave in a similar manner when hybridized with northern individuals (the mean July temperature is 81.1°F and the mean annual precipitation 53.33 inches at Ocala, Florida; the mean July temperature is 82.2°F and the mean annual precipitation 10.64 inches at Barstow, which is near Monahans, Texas). It is probable that natural selection is forming populations with a "low-temperature physiology" in the north and a "high-temperature physiology" in the south. A hybrid between a Texas and Wisconsin *pipiens* might be thought to receive a gene controlled low temperature physiology from one parent and a high temperature physiology from the other parent. Abnormal development would then be due to some sort of physiological unbalance.

There is abundant evidence that the eggs of northern and southern *pipiens* are adapted differently to their special environments. In an earlier study it was found that northern species differ from southern species in a number of embryonic characters such as temperature tolerance, rate of development, size of egg, type of jelly mass, and temperature coefficient of development (MOORE 1939). In most cases the differences were obviously of an adaptive nature. Later it was found that northern populations of *Rana pipiens* differ from southern populations in the same way that northern species differ from southern species (MOORE 1942, 1943, and unpublished observations). Moreover the differences are graded. Wisconsin, Vermont, and New Jersey populations were identical in these characters, Louisiana embryos were better adapted, and the Florida and Texas embryos showed the best adaptation to high temperature.

The question then becomes, would some of these adaptive differences result in hybrid inviability? This question cannot be answered definitely, but the parallel between increasing hybrid inviability and increasing adaptive differences between populations is suggestive.

This concept of evolution involves the synthesis, through the action of natural selection, of adaptive genomes which can incidentally act to isolate the population in which they occur. In many respects this concept is a natural outgrowth of the studies and opinions of students of geographic distribution (MAYR 1942, page 156). Widely distributed species are usually found to show considerable variation. This is especially true if their range encompasses a great diversity of environmental types. If no barriers are present, the variable characters may show a gradient. If barriers are present, the differences between the populations on either side may be more abrupt. In either case the morphological divergence between populations is usually a function of distance or of increasing inaccessability. According to MAYR this divergence of allopatric populations is the principal way speciation occurs in most animal groups. Increasing morphological divergence implies increasing genetic divergence. The latter will almost automatically lead to isolation.

If this type of speciation is at all common, it will help to explain the surprising fact that "taxonomic species" and "genetic species" are usually identical in spite of the entirely different basis for their recognition. In practice a taxono-

mist usually recognizes a species purely on the basis of morphological characters. The geneticist lays emphasis on the lack of interbreeding with other species under natural conditions. If the morphological differences of the taxonomist and the isolating mechanisms of the geneticist arise independently, then taxonomic and genetic species might not coincide. However, if both originate in the same manner, and in some cases probably have the same genetic basis, the usual identity becomes understandable.

In *Rana pipiens* the genetic differences which lead to hybrid inviability are potential isolating mechanisms. Their potential nature should be emphasized, since under natural conditions they are ineffectual. It is of interest, therefore, to consider the conditions which might divide the *Rana pipiens* complex into a northern species and a southern species. This could occur if the intermediate population were destroyed, leaving frogs of the Vermont-Wisconsin type in the north and those of the south Florida-Texas type in the south. This destruction could occur as a result of disease, unsuccessful competition with other species, adverse climatic conditions, submergence of the land, etc. If the depopulated areas were colonized by immigration of the northern and southern types, two very distinct forms would be brought into contact. Crossing between them would result in a wasting of gametes due to the low viability of the hybrids. It would then follow that natural selection would promote the accumulation of additional mechanisms which would further serve to prevent gene exchange between the two types of individuals.

SUMMARY

Rana pipiens from many localities have been used in intraspecific hybridization experiments.

These localities can be arranged in a series that reflects the results of these experiments: (1) Vermont (and Wisconsin), (2) New Jersey, (3) Louisiana, (4) central Florida, (5) southern Florida, (6) Texas. This series also corresponds to the decreasing latitude of the localities, except for Texas.

Hybrids between adjacent members of the series are normal or nearly normal in rate of development and morphology.

In hybrids between progressively more distant members of the series there is a retardation in rate of development, and morphological defects become pronounced. Crosses between the end members of the series result in a high degree of hybrid inviability.

The types of morphological defects follow certain definite patterns. Hybrids derived from northern eggs (or from females from localities low in the series) and southern sperm (or from males from localities high in the series) have enlarged heads and circulatory system defects. Hybrids derived from southern eggs (or from females from localities high in the series) and northern sperm (or from individuals from localities low in the series) have reduced heads with fusion of suckers, fusion of olfactory pits, absence of mouth, and eye abnormalities.

It is thought that evolution in this case involves the synthesis, through the action of natural selection, of adaptive genomes which can incidentally act

to isolate the populations in which they occur. These isolating mechanisms arise, therefore, as a by-product of adaptation.

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A QUANTITATIVE HISTOLOGICAL STUDY OF THE PIGMENT FOUND IN THE COAT-COLOR MUTANTS OF THE HOUSE MOUSE. I. VARIABLE ATTRIBUTES OF THE PIGMENT GRANULES[1]

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AN ANALYSIS of the genetics and physiology of coat color in mammals is very useful as a step toward understanding the processes which go on between original gene action and final character expression. Many studies have been made of genes which affect the whole organism, such as developmental lethals and size factors, including cases in which the effects of genes could be traced back to critical events in certain organs at definite stages in development. In these cases, particularly in mammals, it is difficult enough to discover what tissues are involved in the primary gene action without determining what happens to the metabolism of the cells involved. In contrast, the chain of reactions between gene and character must be relatively short for mammalian coat color genes. The persistence of color in mutant spots and in transplantation experiments indicates that the pigment is determined locally, and the processes concerned continue throughout life, as hair is regularly being shed and replaced. These advantages have been realized by a number of workers, and various types of physiological genetic analyses have been made of the pigments produced in different genotypes of several mammals. Some have been based on observation and grading of colors with the naked eye (LITTLE 1913; WRIGHT 1916, 1917–18, 1925, 1927; DUNN 1936; HADJIDIMITROFF 1933; and HALDANE 1927). Others have based their analyses on chemical isolation of the pigment (DUNN and EINSELE 1938; BAKER and ANDREWS 1944; DANIEL 1938; HEIDENTHAL 1940; E. S. RUSSELL 1939; WRIGHT 1942). SCHILLING (1936) has made a quantitative histological analysis of pigment in the guinea pig, but unfortunately has grouped several genotypes together in some cases, dealing only with grades of color. HARMON and CASE (1941) also have studied guinea pig hair pigment histologically. DANNEEL (1936) has done a genetically accurate histological analysis of rabbit hair pigment, but has not put his results on a quantitative basis. The same is true of LÜHRING'S (1928) analysis of squirrel pigment. HUNT and WRIGHT (1918) give a brief histological study of guinea pig hair pigment. WERNEKE (1916) has done an excellent histological analysis of pigment in the mouse. He describes the granules found in various genotypes, records their distribution between cortex and medulla, and their variation along the hair axis. In general, his conclusions

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as to the effects produced by various genic substitutions still stand, although they, like those of DANNEEL, are not strictly quantitative.

Of the above mentioned authors, only HADJIDIMITROFF and WERNEKE have made any considerable use of one great advantage of hair pigmentation in physiological analysis, one which we feel will contribute greatly to our knowledge of gene action. From tip to base there is laid down an accurate account of the processes going on in a hair bulb throughout its active life. Any variation in pigment-producing activity with time will show up as a difference in amount or type of pigment deposited at different levels of the hair shaft. It is obvious that a histological study of the undisturbed pigment within the hair will provide a perfect place for analysis of this time factor in pigment-gene action.

There are a number of different allelic series of genes in the mouse which act within the hair follicle to affect the number, type, and distribution of pigment granules deposited in the hair as its cells are produced (REED and SANDER 1937; REED 1938a, 1938b; REED and ALLEY 1939). This local gene action may be studied in several ways. While it is occurring, a study may be made of the chemical substances present in the follicles in various genotypes. This has been done for the giunea pig by W. L. RUSSELL, who studied the concentration in a wide variety of genotypes, of an enzyme, dopa-oxidase, which is known to catalyze the change of a tyrosinelike amino acid, l-3,4,-dioxyphenylalanin, to the final hair pigment, melanin. A number of German workers have also studied the enzyme content of the Himalayan rabbit skin during the pigmentation process by a technique called under-cooling (ENGELSMEIER 1935; DANNEEL and SCHAUMANN 1938). LUBNOW (1939) has studied differences in the histological and cytological structure of the matrix-cells of the hair follicle of different genotypes and at different stages in the pigmentation process. Another possible approach is to study the end-product of the pigmentation process which has been deposited in the keratinized hair cells. Spectrophotometric analyses of this hair pigment have been made by DANIEL (1938) for the mouse and by BAKER and ANDREWS (1944) and GINSBURG (1944) for the guinea pig. An analysis of the chemical structure of the pigment would be ideal, but seems impossible at present, especially on the micro-scale necessary to these studies.

However, all of the pigment in mouse hair is granular, and a good deal can be ascertained as to the nature of the various gene actions from counts, measurements, and color determinations of the pigment granules in the cells of the various portions of the hair at successive levels along the hair shaft.

This present paper gives the results of these determinations on various combinations of several major allelic series of coat-color genes in the mouse. Included are the albino series (C, c^{ch}, c^{e}, and c^{a}), which alters the intensity of all colors; the agouti series (A^{y}, A^{w}, and a), which determines the distribution of yellow and sepia along the hair axis; black-brown (B and b), in which the recessive allele changes non-agouti pigment from some shade of black-sepia to a shade of brown; pink-eyed dilution (P and p), in which the recessive allele practically eliminates pigment in the eye and greatly reduces sepia pigment in

the hair; and dilution (*D* and *d*), which reduces the intensity and dulls the shade of all colors. Since this study involves a number of different methods and a wide variety of pigment attributes, it seems most satisfactory to devote the first paper of this series to a description of methods and a simple presentation of the differences found in the various genotypes, saving detailed interpretation of the nature of the changes involved for future publication. The author is very grateful to DR. W. L. RUSSELL for producing the invaluable mouse color stocks used in this investigation. She also wishes to thank both DR. W. L. RUSSELL and MISS LIANE BRAUCH for their many helpful suggestions.

GENERAL METHODS

In order to have a full record of the sequence of events during the pigmentation process in hair growth, it is essential to study whole mounts of representative hairs which have completed their growth. The hair cells are produced from the rapidly dividing matrix cells of the hair bulb (LUBNOW 1939) and very soon become keratinized and pushed above the skin surface. The hair shaft which is thus produced consists of three parts: the outermost coating, or cuticle, a very thin sheath of overlapping scales devoid of pigment; the cortex, a hollow cylinder inside the cuticle, made up of long narrow cells whose longitudinal axes are parallel with that of the hair; and the medulla, or core of the hair, a ladder-like arrangement with horizontal plates (septa) of disk-shaped cells alternating with air-spaces. Four types of hairs have been described in the mouse (DRY 1926) distinguished chiefly by the number of medullary cells per septum. In "lead" hairs there is one cell per septum throughout the length of the hair (the cortex is also especially heavy); these form two per cent of the first pelage (DRY 1926). In "awl" hairs the septa of the middle two-thirds of the hair contain three or possibly four medullary cells. "Auchene" hairs are very similar, except that there is a constriction in the mid-region where only one medullary cell is found in each septum. "Awls" and "auchenes" together constitute 14–15 percent of the first pelage (DRY 1926). The "zig-zag" hairs alternate blades with one medullary cell per septum with short curved constrictions (usually three in number) where only tiny remnants of medullary material remain. These "zig-zag" hairs constitute 83 per cent of the first pelage (DRY 1926). Thus zig-zags and awls or auchenes are the most representative hair types. In all hairs, outgrowth continues until the full length of 0.6–0.7 cm is reached (DRY 1926), after which the hair bulb itself shrinks and becomes keratinized. Fully grown hairs with keratinized bulbs, known as club hairs, can easily be plucked out. They are completely dead, but contain, from tip to base, a consecutive account of what has gone on in a hair bulb during its entire active life.

Club hairs of the first pelage were used in all these studies since their growth has been less influenced by environmental influences than that of later coats. At four or five weeks the first pelage hairs of the crown of the rump have reached the club stage (DRY 1926). Hair samples were plucked from this region

of six animals of each genotype, no more than two of which came from the same litter. The samples were stored in envelopes in darkness until used.

To make whole-mount slides, a small group of hairs were attached to a slide by means of pure egg-white (no glycerin), and after drying were put into each of the following solutions for at least three days: absolute alcohol, alcohol-xylol, and xylol. After this they were imbedded in balsam and covered. This produced preparations free of air-bubbles in the medullary spaces, and giving sharp definition of the unstained pigment granules in the transparent colorless cell matrix. Although glycerin has been used by many workers to remove air from the medullary spaces, its use was avoided here following the discovery by RALPH KELLOGG (unpublished) that glycerin sometimes reacts with the yellow pigment, leading to gradual breakdown of the yellow granules, leaving cells filled with a diffuse yellow coloration. This action has already led to considerable confusion in the literature, apparently accounting for many reports of diffuse non-granular yellow pigment (DANNEEL 1936; LÜHRING 1928; SCHILLING 1936 (slight)).

Individual hairs from each whole mount, usually one zig-zag and one awl or auchene, were studied at a succession of levels from the tip to the base. Distances along the shaft were estimated from the width of the microscopic field (approximately 85μ at 1800×), and generally were indicated simply by using one microscope field as a unit of length. At the very tip of the hair there is a short region with a solid rod of cortex. In zig-zag and awl-auchene hairs the length of this solid tip was found to average four fields (340μ). In order to get a clear measure of the amount of cortical pigment in each genotype, the first level studied in each hair was the most basal field with this solid cortex (arbitrarily called in every case the fourth field). The other levels studied were the 10th, 20th, 30th, 40th, 50th, and 60th fields. At each of the 10th–60th field levels, the number of pigment granules in one medullary cell was recorded. Selection of one medullary cell as a unit for measuring quantity of pigment deposited in the medulla seemed acceptable, since in 11 samples from seven genotypes there were no significant differences in the number of septa per microscopic field. The average number of cells per field (at 900×) was 39 ± 1 for awls (two to three cells per septum in the region counted), 29 ± 1 for auchenes (two cells per septum in the region counted), and 18 ± 1 in zig-zags (one cell per septum). To obtain a comparable measure of cortical pigment along the hair axis, it seemed desirable to determine the number of pigment granules in a volume of cortex approximately equal to that of a single medullary cell. The volume of a cylinder 15μ long from the solid cortex at the tip of the hair was found to be approximately equal to that of the average medullary cell (table 1). In lower regions, the thickness of the cortical wall was found to be 1–2μ, the cross-sectional area approximately twice that of the solid cortical tip, so the volume of the upper half of a 15μ long section in lower regions is sufficiently close to that of a 15μ section of solid cylindrical tip to allow some degree of comparison. Thus at all levels (4th and 10th to 60th at 10-field intervals) the number of pigment granules in a section of cortex 15μ long

(either solid cylinder or upper surface of hollow cylinder) was recorded in addition to the medullary cell counts for the 10th to 60th fields.

These whole mounts were ideal for determining number and variation in number of pigment granules per unit area in cortex and medulla for the various levels of the hair shaft. No significant differences in either cortical or medullary counts were found which might be related to differences among the three hair

TABLE 1

Determination of the length of solid cortical cylinder having volume equal to that of average medullary cell (considered here as a cylindrical disk).

SAMPLE	TYPE HAIR	DIAM. CORTEX IN μ (4TH FIELD)	DIAM. MED. CELL IN μ	HT. MED. CELL IN μ
158	zig-zag	5	6	4
			8	6
	awl	8	10	7
			11	6
139	zig-zag	6	10	6
			12	5
	awl	8	10	8
			10	6
122	zig-zag	6	14	6
	zig-zag	7	12	7
	awl	7	10	8
	awl	7	12	5
116	zig-zag	5	9	5
	zig-zag	5	11	6
	awl	8	13	7
	awl	7	10	6
108	zig-zag	6	12	8
	zig-zag	5	9	6
	awl	8	12	7
	awl	7	8	6
mean (diam.)		6.56	10.45	6.25
mean (rad.)		3.28	5.23	

Mean vol. medullary cell $= \pi r^2 h = \pi(5.23)^2\, 6.25 = 170.94\pi$.
Mean x-section area cortex $= \pi r^2 = \pi(3.28)^2 = 10.76\pi$.

Height cortical cylinder to give vol. equal to med. cell $= \dfrac{170.94\pi}{10.76\pi}$
$= 15.88\mu$

types studied. All of the counts for this paper were made in as short a time as possible in order to avoid possible personal differences in estimation of number of granules in the most crowded types.

Although observations were made of the size, shape, and color of the granules in the whole mounts, no attempt was made to arrive at quantitative indices for these attributes, because the thickness of the cells and the large num-

ber of granules in the more crowded types made detailed observations of individual granules difficult. However, two distinct types of differences in arrangement of granules within the medullary cell were noted. In some genotypes the granules filled the entire cell, while in others they tended to be located more distally, leaving a clear space at the proximal side of each cell. Also, in some types the granules were clumped together, while in others they were arranged more or less evenly. In each genotype the condition with regard to these two attributes, distal arrangement and tendency to clumping, was recorded.

To study the detailed structure of the individual granules, the hair mass remaining from each sample following the making of whole mounts was used for cross sections. These were prepared by the HARDY method (1935) for sectioning fibers. The essential equipment for this method is a small hand microtome, the size of a microscope slide, approximately, which contains a narrow slot into which hairs can be packed as in a vise, and held so tightly that sections cut from the projecting ends must be true cross sections. To operate the microtome, the hair-mass is moved up in the slot by means of a calibrated screw, the projecting hairs are coated with a quick-drying substance, which is allowed to dry, and sections are cut off with a razor blade. Accurate sections as thin as 3–4μ can be obtained by this method. Some tests were made of quick-driers, and collodion in alcohol-ether and methyl-crylate in ether were both found very satisfactory, the methyl-crylate slightly better for thin sections. The sections were affixed to slides by heating slightly, and were then covered with balsam and a cover slip.

The complete outline of individual granules is clearly visible in the cross sections of even the most crowded types. The greatest diameter of 100 granules from each of five samples of each genotype was determined. This diameter was estimated (at 1800×) to the nearest .25μ, using an ocular micrometer, with scale divisions indicating at this magnification almost exactly 1μ (48 ocular units = 50μ). The granules measured were taken from at least three plates of sections from each slide.

The shape of granules in each genotype was also recorded, being listed as round, short oval, long oval, or "irregular shred" (either elongated or roundish). In certain genotypes there were a few scattered pigment bodies very much larger than the others, forming a discrete group well separated from the rest of the size distribution curve; these were called clumps.

The color of the individual granules of each genotype was also determined from the cross sections. To evaluate this, the granules, as seen at 1800×, were matched with the color plates in Ridgway's Color Standards (1912). Since very slight differences in the intensity or color of the light transmitted through the microscope may alter the appearance of the granules, the same lamp, kept in a constant position, was used for all these observations, with the same daylight blue filter and the same setting of the iris diaphragm. The color plates were also always observed with a constant artificial illumination. Perfect matching was difficult, because the granules were seen by transmitted light, the color plates by reflected light. For this reason in many cases two

alternative color names, closely related in Ridgway's classification, were assigned to the same genotype, since it was impossible to say which was the better match. The color plates used fell into three color series, yellow, black-fuscous, and brown. Within the second and third series there were intensity differences in the color plates, varying with genotype, probably differences in concentration of pigments otherwise very similar or identical.

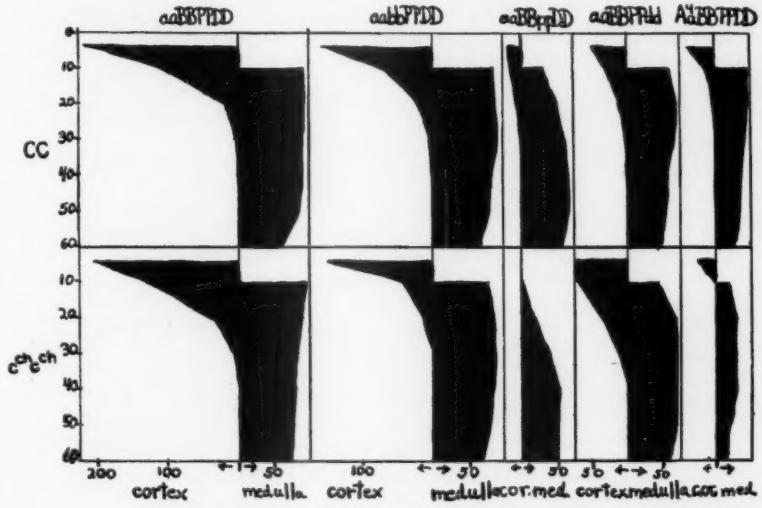

FIGURE 1.—The effect of certain single genic substitutions on cortical and medullary granule number. The figures to the left of the axis represent the mean number of pigment granules per unit volume of cortex at a succession of levels, from the 4th to the 60th field down from the tip of the hair. The figures to the right of the axis represent the mean number of granules per medullary cell at a succession of levels, from the 10th to the 60th field down from the tip of the hair.

The number of granules in a unit area ($25\mu^2$) of a cross section of medullary cell was determined for each genotype tested. Ten unit areas were counted for each of five samples of each genotype. Although these gave no measure of variation along the hair axis, they gave a value for the number of medullary granules in the mid-region of the hair of each genotype, useful for comparison with the more complete counts from whole mounts.

Thus we have observed seven attributes of the pigment granules in each of the 36 genotypes studied: First, attributes determined primarily from whole mounts: (1) Number of medullary granules per medullary cell, counted at 10-field intervals along the hair axis confirmed for the mid-region by independent counts from sections. (1a) (Corollary) Tendency toward a lag in the number per medullary cell produced near tip of hair. (2) Number of cortical granules per unit volume equal to that of average medullary cells. (3)

Tendency to distal arrangement of granules within medullary cells. Second, attributes determined primarily from cross sections: (4) Size of granules (greatest diameter). (5) Shape of granules. (6) Clumping of granules (also checked from whole mounts). (7) Color as matched against Ridgway's Color Standards. (7a) Intensity of color.

TABLE 2

The mean number of granules at successive levels in the non-agouti genotypes tested. (Cort. = granules/unit cortical volume; med. = granules/medullary cell; M = mean; E = standard error; field = width field at 1800×, approx. 85μ).

GENOTYPE	REGION	FIELD											
		4TH		10TH		20TH		30TH		40TH		50TH		60TH	
		M	E	M	E	M	E	M	E	M	E	M	E	M	E
aaCCBBPPDD	cort.	219	±7	113	±12	21	±5	4	±3	2	±2	0		0	
	med.			93	±14	95	±11	86	±2	90	±6	87	±2	61	±3
aac^{ch}c^{ch}PPBBDD	cort.	203	±8	128	±9	35	±4	8	±3	0		0		0	
	med.			98	±2	93	±3	85	±3	80	±3	77	±5	73	±2
aac^{e}c^{e}BBPPDD	cort.	20	±4	2	±2	0		0		0		0		0	
	med.			19	±2	38	±3	42	±3	38	±2	38	±2	33	±3
aac^{e}c^{e}BBPPDD	cort.	0		0		0		0		0		0		0	
	med.			0		0		0		0		0		0	
aaCCbbPPDD	cort.	160	±8	73	±3	21	±10	4	±3	0		0		0	
	med.			82	±3	90	±4	95	±4	85	±4	82	±8	70	±8
aac^{ch}c^{ch}bbPPDD	cort.	147	±5	44	±8	14	±5	1	±1	0		0		0	
	med.			79	±3	83	±3	88	±3	87	±4	81	±5	71	±5
aac^{e}c^{e}bbPPDD	cort.	31	±3	2	±2	1	±1	0		0		0		0	
	med.			20	±2	34	±2	50	±4	52	±5	39	±5	28	±4
aaCCBBppDD	cort.	18	±3	15	±1	4	±2	0		0		0		0	
	med.			32	±2	51	±3	62	±4	71	±5	69	±5	59	±5
aac^{ch}c^{ch}BBppDD	cort.	0		0		0		0		0		0		0	
	med.			1	±1	19	±4	40	±3	55	±4	51	±4	52	±4
aac^{e}c^{e}BBppDD	cort.	0		0		0		0		0		0		0	
	med.			0		0		0		0		0		0	
aaCCBBPPdd	cort.	49	±4	31	±3	12	±3	4	±2	0		0		0	
	med.			59	±5	73	±5	70	±5	58	±5	57	±6	52	±9
aac^{ch}c^{ch}BBPPdd	cort.	72	±15	71	±10	35	±11	11	±4	0		0		0	
	med.			47	±6	71	±3	72	±3	73	±5	70	±3	58	±8
aaCCbbppDD	cort.	17	±3	11	±3	1	±1	0		0		0		0	
	med.			30	±2	43	±2	47	±3	60	±4	59	±3	51	±4
aaCCbbPPdd	cort.	41	±5	29	±5	13	±4	5	±2	0		0		0	
	med.			40	±4	47	±4	53	±4	52	±7	49	±5	51	±7
aaCCBBppdd	cort.	0		0		2	±2	0		0		0		0	
	med.			9	±3	34	±3	48	±3	50	±4	59	±4	49	±4
aaCCbbppdd	cort.	0		0		0		0		0		0		0	
	med.			7	±3	32	±4	43	±3	47	±4	53	±5	42	±1

RESULTS

As stated in the description of methods, the number of both cortical and medullary granules in a unit volume was counted at successive levels in whole mounts of 12 hairs (six samples) of each of the 36 genotypes tested. The results for the non-agouti types are given in table 2, for yellows in table 3. The mean number of cortical granules per unit volume varies from 219±7 for the 4th field of *aaCCBBPPDD* hairs down to zero for albinos and certain pink-eyed types. The mean number of granules per medullary cell varies from 90–100

TABLE 3

The mean number of granules at successive levels in the yellow genotypes tested. (Cort. = granules/unit cortical volume; med. = granules/medullary cell; M = mean; E = standard error; field = width field at 1800×, approximately 85μ.)

GENOTYPE	REGION	FIELD											
		4TH		10TH		20TH		30TH		40TH		50TH		60TH	
		M	E	M	E	M	E	M	E	M	E	M	E	M	E
A^{v}aCCBBPPDD	cort.	39	±7	12	±4	3	±2	0		0		0		0	
	med.			47	±2	47	±2	42	±2	40	±3	35	±3	29	±3
A^{v}ac^{ch}c^{ch}BBPPDD	cort.	25	±7	0		0		0		0		0		0	
	med.			22	±2	30	±1	30	±3	29	±3	20	±2	18	±2
A^{v}ac^{e}c^{e}BBPPDD	cort.			0		5	±3	0		0		0		0	
	med.					0		0		0		0		0	
A^{v}aCCbbPPDD	cort.	35	±3	19	±4	2	±1	0		0		0		0	
	med.			43	±3	43	±2	40	±2	34	±2	33	±2	31	±2
A^{v}ac^{ch}c^{ch}bbPPDD	cort.	23	±4	0		0		0		0		0		0	
	med.			18	±1	24	±2	24	±2	24	±2	22	±1	16	±1
A^{v}ac^{e}c^{e}bbPPDD	cort.	0		0		0		0		0		0		0	
	med.			0		1	±1	0		0		0		0	
A^{v}aCCBBppDD	cort.	14	±4	5	±3	0		0		0		0		0	
	med.			41	±2	45	±3	41	±3	35	±3	38	±2	34	±3
A^{v}ac^{ch}c^{ch}BBppDD	cort.	0		0		0		0		0		0		0	
	med.			13	±2	23	±4	20	±3	19	±3	22	±3	17	±2
A^{v}aCCBBPPdd	cort.	8	±3	2	±1	1	±1	0		0		0		0	
	med.			33	±4	41	±4	38	±3	41	±3	32	±3	24	±2
A^{v}ac^{ch}c^{ch}BBPPdd	cort.	5	±3	0		0		0		0		0		0	
	med.			11	±3	15	±3	16	±3	16	±3	14	±3	9	±2
A^{v}aCCbbppDD	cort.	15	±2	3	±2	0		0		0		0		0	
	med.			44	±2	37	±2	38	±2	38	±4	36	±3	24	±3
A^{v}aCCbbPPdd	cort.	5	±2	0		0		0		0		0		0	
	med.			29	±2	33	±3	32	±3	28	±5	27	±3	31	±6
A^{v}aCCBBppdd	cort.	0		0		0		0		0		0		0	
	med.			31	±4	44	±3	42	±4	38	±3	32	±2	36	±4
A^{v}aCCbbppdd	cort.	0		2	±2	0		0		0		0		0	
	med.			29	±2	39	±3	39	±4	42	±3	36	±3	42	±6

in the most intense non-agouti types down to zero in all albinos and certain extreme dilute types. The number of granules per unit volume varies along the length of the hair in different ways under different conditions. In general, heavy cortical pigment is found only near the tip, and only in certain very intensely colored types. It drops out irregularly in the mid-region of the hair. In certain other lightly colored types, no cortical pigment is produced. This

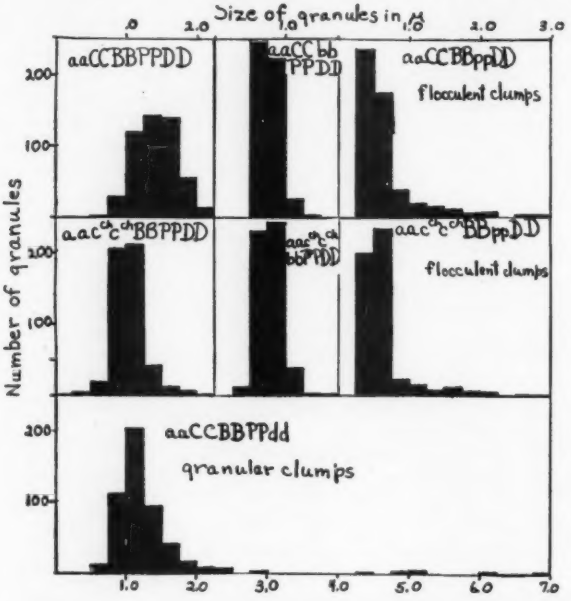

FIGURE 2.—Distribution of granule sizes in seven representative non-agouti genotypes. The total for each genotype is 500 granules; their greatest diameters are measured to the nearest 0.25μ interval.

is usually associated with a hesitation in the commencement of pigmentation in the medulla, likewise found only in lightly pigmented types (in intensely colored types the medullary pigment is evenly distributed along the length of the hair). We have called this tendency to smaller numbers of medullary pigment granules near the tip of the hair "pigmentation lag." Figure 1 shows graphically the amount and type of variation in granule counts found along the axis of ten different genotypes.

One further set of granule counts from whole mounts should be recorded here. Slides were made of six samples from each of six different light-bellied agouti genotypes. The means of the counts of these slides at successive levels are given in table 4. In each case, number (and also size, shape, and color of

granules, not listed here) of the granules in the agouti corresponds better with the non-agouti partner at the tip, then between fields six or seven and 15 to 18, with the yellow partner, then throughout the remainder of the hair length, with the non-agouti again.

TABLE 4

The mean number of granules at successive levels in six agouti genotypes. (Cort. = granules/unit cortical volume; med. = granules/medullary cell; M = mean; E = standard error; field = width at 1800×). The double vertical lines mark off the measurements corresponding with yellow.

GENOTYPE	REGION	FIELD													
		4TH		10TH		20TH		30TH		40TH		50TH		60TH	
		M	E	M	E	M	E	M	E	M	E	M	E	M	E
A^{w}A^{w}CCBBPPDD	cort.	201	±6	11	±6	26	±3	23	±4	0		0		0	
	med.			43	±3	99	±1	88	±3	95	±3	95	±3	93	±1
A^{w}A^{w}c^{e}c^{e}BBPPDD	cort.	50	±6	0		0		0		0		0		0	
	med.			3	±2	34	±4	39	±2	37	±3	44	±3	32	±2
A^{w}A^{w}CCbbPPDD	cort.	150	±10	12	±3	18	±9	0		0		0		0	
	med.			45	±2	85	±5	89	±5	82	±5	82	±4	86	±7
A^{w}A^{w}c^{e}c^{e}bbPPDD	cort.	38	±4	0		0		0		0		0		0	
	med.			2	±1	32	±5	51	±4	58	±4	62	±4	52	±4
A^{w}A^{w}CCBBppDD	cort.	49	±7	8	±2	3	±2	0		0		0		0	
	med.			52	±2	65	±4	78	±3	80	±4	88	±5	77	±3
A^{w}A^{w}c^{ch}c^{ch}BBppDD	cort.	0		0		0		0		0		0		0	
	med.			19	±2	32	±4	41	±4	56	±4	49	±4	38	±4

Description of individual medullary granules found in the various genotypes, as determined from cross sections, are given in table 5. The table includes for each genotype the matching with Ridgway's Color Standards, remarks on the granule shape, and the mean and standard error of the measurements of the greatest diameter of 500 granules from the cross sections of five different samples. The colors fall into three series, the aniline-yellow (or raw-sienna) series of the *A^{y}a* genotypes, the black-fuscous series of the *aaBB* genotypes, and the carob-mummy brown series of the *aabb* genotypes. There are three grades of intensity in the black-fuscous series, grading down from intense black to fuscous black (or deep blackish-brown 3), and finally to fuscous (or dusky drab). There are two grades of intensity in the brown series, grading down from carob-brown (or chestnut-brown) to mummy brown (or raw umber). There is only one grade of intensity in the aniline-yellow series. The shape varies from long ovals for *aaCCBBPPDD* through shorter ovals, spheres, to irregular shred-like shapes which may be either elongated or roundish. The mean greatest diameter of medullary granules varies from 1.44 ± .013μ for *aaCCBBPPDD* down to 0.61 ± .013μ for *aaCCbbppDD*. Figure 2 shows graphically the distribution of the diameter measurements in seven representative

non-agouti genotypes, and demonstrates that there is considerable variation both in means and in type of distribution. The narrowest range of granule sizes is in full color brown, *aaCCbbPPDD*, where it is only 1.0μ, from 0.5 to 1.5μ. The widest range in one genotype is found in dilute black (*aaCCBBPPdd*) where it is 6.5μ, from 0.5μ up to 7.0μ. Genotypes with clumping such as will be described in the next section tend to have skewed distributions of granule size with a few very large pigment bodies. No distributions for yellow genotypes are given graphically here, since they show little variation among the genotypes tested.

TABLE 5

Description of individual granules, taken from cross sections, in all of the genotypes tested. The measurements are means of the greatest diameters of 500 granules from five samples of each type, graded by 0.25μ intervals. The colors are evaluated by matching with Ridgway's color charts. The shape is the usual one for each genotype, recorded for each sample at the time of measuring.

GENOTYPE	COLOR	SHAPE	DIAMETER
aaCCBBPPDD	intense black	long oval	1.44 ± .013
aac^{ch}c^{ch}BBPPDD	fuscous black	oval	1.05 ± .010
aac^{c}c^{c}BBPPDD	fuscous	round	0.94 ± .014
aaCCbbPPDD	carob-brown	round	0.77 ± .007
aac^{ch}c^{ch}bbPPDD	carob-brown	round	0.79 ± .008
aac^{c}c^{c}bbPPDD	carob-brown	round	0.77 ± .012
aaCCBBppDD	fuscous	long shreds	0.64 ± .018
aac^{ch}c^{ch}BBppDD	fuscous	long shreds	0.62 ± .015
aaCCBBPPdd	fuscous black	oval	1.23 ± .011
aac^{ch}c^{ch}BBPPdd	fuscous black	oval	1.12 ± .025
aaCCbbppDD	mummy-brown	short shreds	0.61 ± .013
aaCCbbPPdd	carob-brown	round	0.98 ± .020
aaCCBBppdd	fuscous	long shreds	0.73 ± .022
aaCCbbppdd	mummy brown	short shreds	0.67 ± .023
A^{y}aCCBBPPDD	aniline yellow	round	0.83 ± .013
A^{y}ac^{ch}c^{ch}BBPPDD	aniline yellow	round	0.77 ± .009
A^{y}aCCbbPPDD	aniline yellow	round	0.82 ± .010
A^{y}ac^{ch}c^{ch}bbPPDD	aniline yellow	round	0.76 ± .010
A^{y}aCCBBppDD	aniline yellow	round	0.80 ± .011
A^{y}ac^{ch}c^{ch}BBppDD	aniline yellow	round	0.66 ± .010
A^{y}aCCBBPPdd	aniline yellow	round	0.79 ± .012
A^{y}ac^{ch}c^{ch}BBPPdd	aniline yellow	round	0.77 ± .017
A^{y}aCCbbppDD	aniline yellow	round	0.79 ± .011
A^{y}aCCbbPPdd	aniline yellow	round	0.82 ± .015
A^{y}aCCBBppdd	aniline yellow	round	0.81 ± .013
A^{y}aCCbbppdd	aniline yellow	round	0.82 ± .017

Table 6 records the observations from both whole mounts and sections on the two remaining granule attributes, tendency to distal arrangement and tendency to clumping. Three grades of distal arrangement are distinguished: "none," indicating that the entire cell is evenly filled with pigment granules; "slight," indicating a small proximal space clear of pigment granules; and

TABLE 6

Description of granule arrangement within the medullary cells of the genotypes tested.

GENOTYPES	DISTAL ARRANGEMENT	CLUMPING
aaCCBBPPDD	none	none
aac^{ch}c^{ch}BBPPDD	none	none
aac^{e}c^{e}BBPPDD	cap	none
aaCCbbPPDD	none	none
aac^{ch}c^{ch}bbPPDD	none	none
aac^{e}c^{e}bbPPDD	cap	none
aaCCBBppDD	slight	flocculent
aac^{ch}c^{ch}BBppDD	cap	flocculent
aaCCBBPPdd	slight	granular
aac^{ch}c^{ch}BBPPdd	slight	granular
aaCCbbppDD	cap	flocculent
aaCCbbPPdd	slight	granular
aaCCBBppdd	cap	flocculent and granular
aaCCbbppdd	cap	flocculent and granular
A^{v}aCCBBPPDD	cap	none
A^{v}ac^{ch}c^{ch}BBPPDD	cap	none
A^{v}aCCbbPPDD	cap	none
A^{v}ac^{ch}c^{ch}bbPPDD	cap	none
A^{v}aCCBBppDD	cap	none
A^{v}ac^{ch}c^{ch}BBppDD	cap	none
A^{v}aCCBBPPdd	cap	granular
A^{v}ac^{ch}c^{ch}BBPPdd	cap	granular
A^{v}aCCbbppDD	cap	none
A^{v}aCCBBppdd	cap	granular
A^{v}aCCbbppdd	cap	granular

"cap," indicating that the greater part of the cell is clear with the pigment confined to a narrow distal cap. In the "slight" and "cap" types there is in addition to the proximal clear space another localization of the pigment granules in certain regions of the hair. In the middle sections of zig-zags, between the first and third bending constrictions, the medullary cells usually contain two distinct rows of granules, above and below a clear space which seems to be a rigid bar across the middle of the cell.

Two qualitatively different states are recorded in the "clumping" column of table 6; "flocculent" type and "granular" type. In the first there are loose flocculent masses, often as big as 3μ in diameter, which cannot easily be determined to be composed of clumped granules. In the granular type there are large sharply defined clumps which may quite possibly be composed of the regular granules of the genotype concerned cohering to each other. Points of deeper color, resembling individual granules, are often seen within the medullary cells but occasionally form separate entities attached to the inner wall of the cortical cylinder. This type of clump is often very large and conspicious,

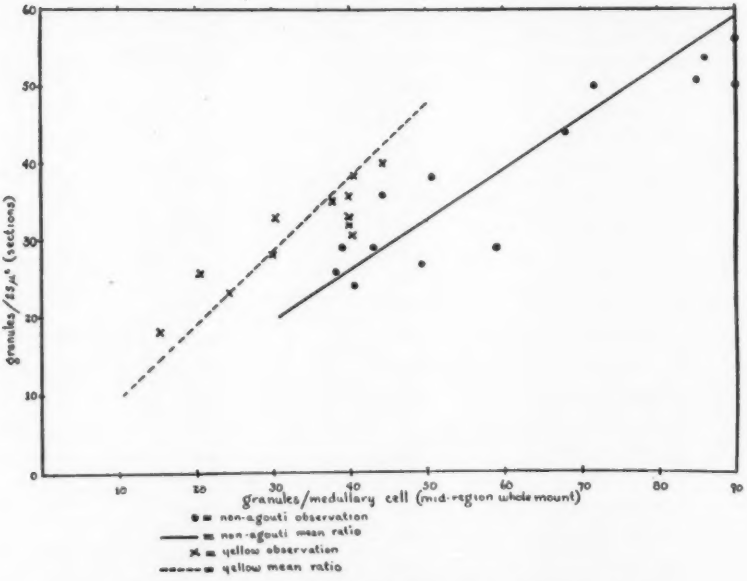

FIGURE 3.—Ratio of granule counts from cross-sections to counts from the mid-region of whole-mounts.

up to 7μ in diameter, and may be seen at magnifications considerably lower than those used in this study. Both types of clumps are very few in number relative to the smaller granules, as can be seen from the distributions in figure 2. In the types which have granular clumping many of the granules which are not actually clumped are somewhat irregularly arranged, giving the hair a spotty appearance at low magnifications. This irregularity and the large size of granular clumps make it possible to distinguish definitely between certain flocculently-clumped genotypes which also have granular clumping, and others which are similar except that they have no granular clumps.

Although the difficulties of making granule counts from cross sections, where the exact location on the hair axis cannot be controlled, have been mentioned, estimates have been made for all the genotypes tested and have led to results worth reporting. These cross-section counts are especially welcome, because they give independent confirmation of certain whole-mount findings such as essential identity of granule number in certain different genotypes (*aaCCBBPPDD*, *aa$c^{ch}c^{ch}$BBPPDD*, *aaCCbbPPDD*, and *aa$c^{ch}c^{ch}$bbPPDD*, for example). Table 7 and figure 3 give the mean of ten counts of the number of granules in a $25\mu^2$ section of a cross section of a medullary cell in each genotype tested, and compare these values with those obtained from the whole-mount counts. Since it seems that all of the sections must be taken from levels corresponding to the region of whole hairs lying between the 15th and 45th fields, the value used in comparing the whole mounts with the sections was the mean of the number of granules per medullary cell, combining counts from the 20th, 30th, and 40th fields. The non-agouti types show a fairly constant ratio of section to whole-mount value, the mean ratio being $0.65 \pm .02$, and only one value, 0.82 for *aaCCBBppdd*, differing from the mean of means by more than twice the standard error of the difference. The yellow types also show a fairly constant ratio of section to whole-mount count, although the mean ratio, $0.95 \pm .04$, is very different from that found for non-agoutis. This difference must be due at least in part to the extreme tendency for the granules of yellow to be concentrated in the distal portion of the cell, so that a fully pigmented area of $25\mu^2$ will contain most of the granules from an entire cell. In the yellows, means of three genotypes, *A^{v}a$c^{ch}c^{ch}$BBppDD*, *A^{v}a$c^{ch}c^{ch}$BBPPdd*, and *A^{v}aCCBBppdd*, differ from the mean of means by more than twice the standard error of the difference. Considering the amount of variation in pigment distribution along the hair axis and within the cells, the observed variations in ratio are not surprising, and the results of cross-section counts are sufficiently accurate to help in interpretation of results from the more accurate whole-mounts, particularly in cases where the whole-mounts are very crowded.

The results given in this paper have shown clearly that mouse coat color differences cannot arise merely from differences in diffraction from different sized pigment granules, as has been suggested as a possibility (DUNN and EINSELE 1938; DANIEL 1938). Table 8 shows that variations in many factors may lead to color changes. In certain cases there are differences in granule size which account for the entire difference in appearance between two genotypes, such as the black *aaCCBBPPDD* (mean greater diameter $1.44 \pm .013\mu$, granules/medullary cell, mid-region 90 ± 2) and the dark sepia *aa$c^{ch}c^{ch}$BBPPDD* (mean greater diameter $1.05 \pm .010\mu$, granules/medullary cell, mid-region 86 ± 2). The difference in appearance is not nearly as great here, however, as that found in other cases where granule size is identical. For instance, the mean size of granules in *A^{v}a$c^{ch}c^{ch}$BBPPDD* is $0.77 \pm .009\mu$, and the mean size in *aaCCbbPPDD* is $0.77 \pm .007\mu$; yet one is cream, the other dark brown. The aniline yellow color of the granules in *A^{v}a$c^{ch}c^{ch}$BBPPDD* and the carob-brown color of granules in *aaCCbbPPDD* appear to be much more pertinent

to the difference in general appearance than do the granule sizes. In this case, however, there is quite a difference between the types in number of granules, which undoubtedly contributes to the difference in appearance. Let us com-

TABLE 7

Medullary granule counts from unit areas ($25\mu^2$) of cross sections of genotypes tested, and their comparison with whole-mount counts from the corresponding mid-region (20th, 30th, 40th fields).

GENOTYPE	SECTIONS GRANULES/$25\mu^2$ M	E	WHOLE-MOUNT GRANULES/MED. CELL M	E	RATIO SECTIONS/WH. M. M	E
aaCCBBPPDD	50	±2	90	±2	.56	±.03
aac^{ch}c^{ch}BBPPDD	54	±2	86	±2	.63	±.03
aac^{c}c^{c}BBPPDD	29	±1	39	±2	.74	±.05
aaCCbbPPDD	56	±1	90	±2	.62	±.02
aac^{ch}c^{ch}bbPPDD	52	±1	85	±2	.61	±.02
aac^{c}c^{c}bbPPDD	29	±1	43	±2	.67	±.04
aaCCBBppDD	29	±1	59	±2	.49	±.02
aac^{ch}c^{ch}BBppDD	26	±1	38	±3	.68	±.06
aaCCBBPPdd	44	±1	68	±3	.65	±.03
aac^{ch}c^{ch}BBPPdd	50	±1	72	±2	.69	±.02
aaCCbbppDD	27	±1	49	±2	.55	±.03
aaCCbbPPdd	38	±1	51	±3	.75	±.05
aaCCBBppdd	36	±1	44	±2	.82	±.04*
aaCCbbppdd	24	±1	41	±2	.59	±.04
mean of non-agouti ratios					.65	±.02
A^{v}aCCBBPPDD	40	±1	44	±2	.91	±.05
A^{v}ac^{ch}c^{ch}BBPPDD	28	±1	30	±1	.93	±.05
A^{v}aCCbbPPDD	36	±1	40	±2	.90	±.05
A^{v}ac^{ch}c^{ch}bbPPDD	23	±1	24	±1	.96	±.06
A^{v}aCCBBppDD	38	±1	41	±2	.93	±.05
A^{v}ac^{ch}c^{ch}BBppDD	26	±1	21	±2	1.24	±.10*
A^{v}aCCBBPPdd	32	±1	40	±2	.80	±.04
A^{v}ac^{ch}c^{ch}BBPPdd	18	±1	16	±2	1.13	±.02*
A^{v}aCCbbppDD	35	±1	38	±2	.92	±.05
A^{v}aCCbbPPdd	33	±1	31	±2	1.06	±.08
A^{v}aCCBBppdd	31	±1	41	±2	.76	±.04*
A^{v}aCCbbppdd	33	±1	40	±2	.83	±.05
mean of all yellow ratios					.95	±.04

* Differs from the mean of means for non-agoutis or yellows by more than twice the standard error of the difference.

pare *A^{v}aCCBBPPDD* (mean greater diameter of 0.83 ± .013μ, granules/medullary cell, mid-region 44 ± 2) with *aac^{e}c^{e}bbPPDD* (mean greater diameter 0.77 ± .008μ, granules/medullary cell, mid-region, 43 ± 2). Here both number and size of granules are very close in the two types, yet one appears yellow, the other light brown. The important fact here seems to be that the individual granules in the yellow type are aniline yellow, while those in the extreme dilute brown are carob-brown. We can also pick out cases where a difference in appearance is due entirely to a difference in number of granules. Let us compare two types which look just alike, *aaCCbbPPDD* (mean greater diameter

TABLE 8

Analysis of differences in granule attributes responsible for observed coat-color differences, demonstrating that these cannot be due merely to changes in granule size, but to different factors under different conditions.

		SIZE DOES IT		
aaCCBBPPDD	size	1.44 ± .013μ	gr./med. cell	90 ± 2
aac^{ch}c^{ch}BBPPDD	size	1.05 ± .010μ	gr./med. cell	86 ± 2
		COLOR DOES IT (AIDED BY NUMBER)		
A^{v}ac^{ch}c^{ch}BBPPDD	size	0.77 ± .009μ	color yellow (looks cream)	28 ± 1
aaCCbbPPDD	size	0.77 ± .007μ	color carob brown (brown)	56 ± 1
		COLOR ALONE DOES IT		
A^{v}aCCBBPPDD	size	0.83 ± .013μ	gr./med. cell	44 ± 2 (yellow)
aac^{e}c^{e}bbPPDD	size	0.77 ± .008μ	gr./med. cell	43 ± 2 (brown)
		NUMBER ALONE DOES IT		
aaCCbbPPDD	size	0.77 ± .007μ	gr./med. cell	90 ± 2 (dark)
aac^{ch}c^{ch}bbPPDD	size	0.79 ± .008μ	gr./med. cell	85 ± 2 (dark)
aac^{e}c^{e}bbPPDD	size	0.77 ± .012μ	gr./med. cell	43 ± 2 (light)

0.77 ± .007μ, granules/medullary cell, mid-region, 90 ± 2) and *aac^{ch}c^{ch}bbPPDD* (mean greater diameter 0.79 ± .008μ, granules/medullary cell, mid-region, 85 ± 2), with a third which differs from them only in granule number, *aac^{e}c^{e}bbPPDD* (mean greater diameter, 0.77 ± .012, granules/medullary cell, mid-region, 43 ± 2). The first two of these types are dark brown, the third light brown. The pigment granules in all three are not only identical in size, they are all carob-brown. Thus only the difference in granule number can account for the change in appearance with this extreme-dilute substitution. The other granule attributes which we have studied have all undoubtedly contributed to the general coat color, although we cannot pick out any case where one of these is the sole cause of a difference in appearance; granule shape is important (particularly in pink-eyes), also arrangement of granules (especially the clumping of dilutes), and pigmentation lag (particularly in pink-eyes).

So we have an overall picture of a large number of different variable pigmentation attributes contributing to the differences in appearance of these coat-color mutants of the house mouse. In later papers we will discuss the interrelations of these attributes and attempt to determine the factors responsible for their observed variations.

SUMMARY

A quantitative histological analysis has been made of the pigment granules in representative mouse hairs from the first coat of animals carrying various combinations of the following allelic series of genes: albino series (*C*, c^{ch}, c^{e}, and c^{a}), agouti series (A^{w}, A^{y}, and *a*), black-brown, (*B*, *b*), pink-eye dilution (*P*, *p*), and dilution (*D*, *d*). The materials for these studies were whole-mounts, prepared with alcohol, alcohol-xylol, and xylol, and mounted in balsam, and cross sections, prepared by the Hardy method, using methyl-crylate as a coating substance, and mounted in balsam.

The granule attributes found to vary among the various genotypes were: (1) The number of granules per medullary cell, counted at 10-field intervals along the axis of whole-mount hairs. The mean for genotypes varied from 98 ± 2 for the 10th field of *aac*^{ch}*c*^{ch}*BBPPDD* down to zero in all albinos and certain extreme dilute types. In some types there is a tendency to variation in the number of granules per medullary cell at different points along the hair axis. (2) The number of cortical granules per unit volume equal to that of an average medullary cell. The cortical granules are generally found only near the tip of the hair, and the mean number at the 4th field varied from 219 ± 7 for *aaCCBBPPDD* down to zero for all albinos, unpigmented extreme dilutes, and certain pink-eyed types. (3) The tendency to distal arrangement of granules within medullary cells. In the most intensely colored types the granules fill the entire cell, but in intermediate types there is a small clear space at the proximal side of the cell, and in lightly colored types this clear space extends so far that all the pigment is located in a small distal cap. (4) Size of granules in medullary cells. The greatest diameter of 500 granules from each genotype showed that the mean size varied from $1.44 \pm .013\mu$ for *aaCCBBPPDD* down to $0.61 \pm .013\mu$ for *aaCCbbppDD*. The range of particle size also varies with genotype. The narrowest range, in *aaCCbbPPDD*, is 1μ from 0.5–1.5μ greater diameter; the widest, in *aaCCBBPPDD*, is 6.5μ, from 0.50μ–7.00μ greater diameter. In all types with a very wide range of granule sizes, the distributions are very skew, with a few particles (clumps) near the upper limit. (5) Shape of granules in medullary cells. Four basic granule shapes are found characterizing the various genotypes: long oval, oval, round, and irregular shred-shaped. (6) The clumping of granules. Two types of clumps are found in certain of the genotypes: loose flocculent masses (up to 3μ) and sharply defined granular clumps (up to 7μ diameter). (7) The color of pigment granules. These fall into three color series; (I) the yellow series, with only one grade of intensity, all granules matching with the aniline yellow or raw-sienna swatches in Ridgway's Color Standards; (II) the black fuscous series, with three grades of intensity, of which pure black is the darkest, fuscous black (or blackish-brown-3) is intermediate, and fuscous (or dusky drab) is lightest; and (III) the brown series, with two grades of intensity, of which carob-brown (or chestnut-brown) is the darker, and mummy-brown (or raw umber) the lighter.

Differences in all of these variable granule attributes have been shown to

make important contributions to the final coat color differences among the 36 genotypes observed in this paper.

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SUMMARIES FROM FORTHCOMING PAPERS

BLANC, RICHARD. *Domininigenes of the vestigial series in Drosophila melanogaster.*—Received January 26, 1946.—Reanalysis of GOLDSCHMIDT'S vestigial-dominigene (*Doho*) stock has indicated segregation in Doho for one or more otherwise unidentified dominance modifiers of vestigial.

GARDNER'S results from outcrosses of Doho with normal stocks have been shown to be compatible with homozygosity of Doho for all three of GOLDSCHMIDT'S dominigenes, thereby obviating GARDNER'S assumption of balanced heterozygosity for one of these dominigenes.

The demonstration of the ubiquity of dominigenes in wild type stocks has led to the construction of isochromosomal stocks for use in further crosses.

The observed interaction of GOLDSCHMIDT'S second (*B*) and third chromosome (*A*) dominigenes indicates that the same or closely related developmental processes are affected.

It has been further observed that *B* is effective in altering the dominance of vestigial in the absence of *A*, and *vice versa*, and that *B* exceeds *A* in effect on penetrance of wing notching in *vg*-heterozygotes.

The heightened notching penetrance in the presence of vestigial from the standard UNIVERSITY OF CALIFORNIA stock is considered in the light of possible introduction of further dominigenes from the standard stock and the question of isoallelism in the vestigial series.

Further crosses with a number of vestigial stocks has demonstrated the presence of additional dominigenes in most of these stocks.

GOLDSCHMIDT'S and HOENER'S investigation of the effect of the dominigenes on alleles of the vestigial series has been amplified by the use of further alleles, as well as deficiencies, of vestigial.

Further consideration of the dominigenes has led to the partial identification of GOLDSCHMIDT'S second chromosome "gene" *B* with *pr* (purple) and third chromosome "gene" *A* with *ss* (spineless).

GOLDSCHMIDT'S identification of the X-chromosome dominigene *do-X* as an allele of *ct* (cut) has been shown to be inconclusive.

Demonstration of the ubiquity of "unseen" dominance modifiers has led to a criticism of GARDNER'S conclusions regarding the dominigene action of a number of point mutants.

MATHER'S distinction between polygenes and oligogenes has been disputed on the basis of the parallelisms between dominigenes and polygenes and the apparent identify of *B* and *A* with the visible mutants *pr* and *ss*.

A hypothesis involving alteration in time relationships of developmental processes has been presented as a tentative explanation of the mode of action of the dominigenes.

VILLEE, CLAUDE A. *Phenogenetic studies of the homoeotic mutants of Drosophila melanogaster.* IV. *Homoeotic and "growth rate" genes.*—Received Febru-

ary 10, 1946.—Isogenic stocks of the growth rate genes dachs, dachsous, four-jointed, and combgap, and the homoeotic genes proboscipedia, aristapedia, aristapedia-Bridges, and bithorax were set up, all possible combinations of growth rate and homoeotic genes were made and the phenotypes of the combinations noted.

Aristapedia and aristapedia-Bridges decrease the number of segments per tarsus and the length of the individual tarsal segments when combined with *d*, *ds*, *fj*, or *cg*. Bithorax decreases the number of segments per tarsus in combination with dachs and four-jointed but not in combination with dachsous and combgap.

Dachs has the same effect on the phenotypes of the homoeotic mutants as exposing the larvae of homoeotic flies to low temperature. Four-jointed, dachsous and combgap have an effect similar to that of exposing the larvae to high temperature. This corroborates and extends WADDINGTON's finding that dachs causes a lessening and combgap an increase in the rate of development during the larval period.

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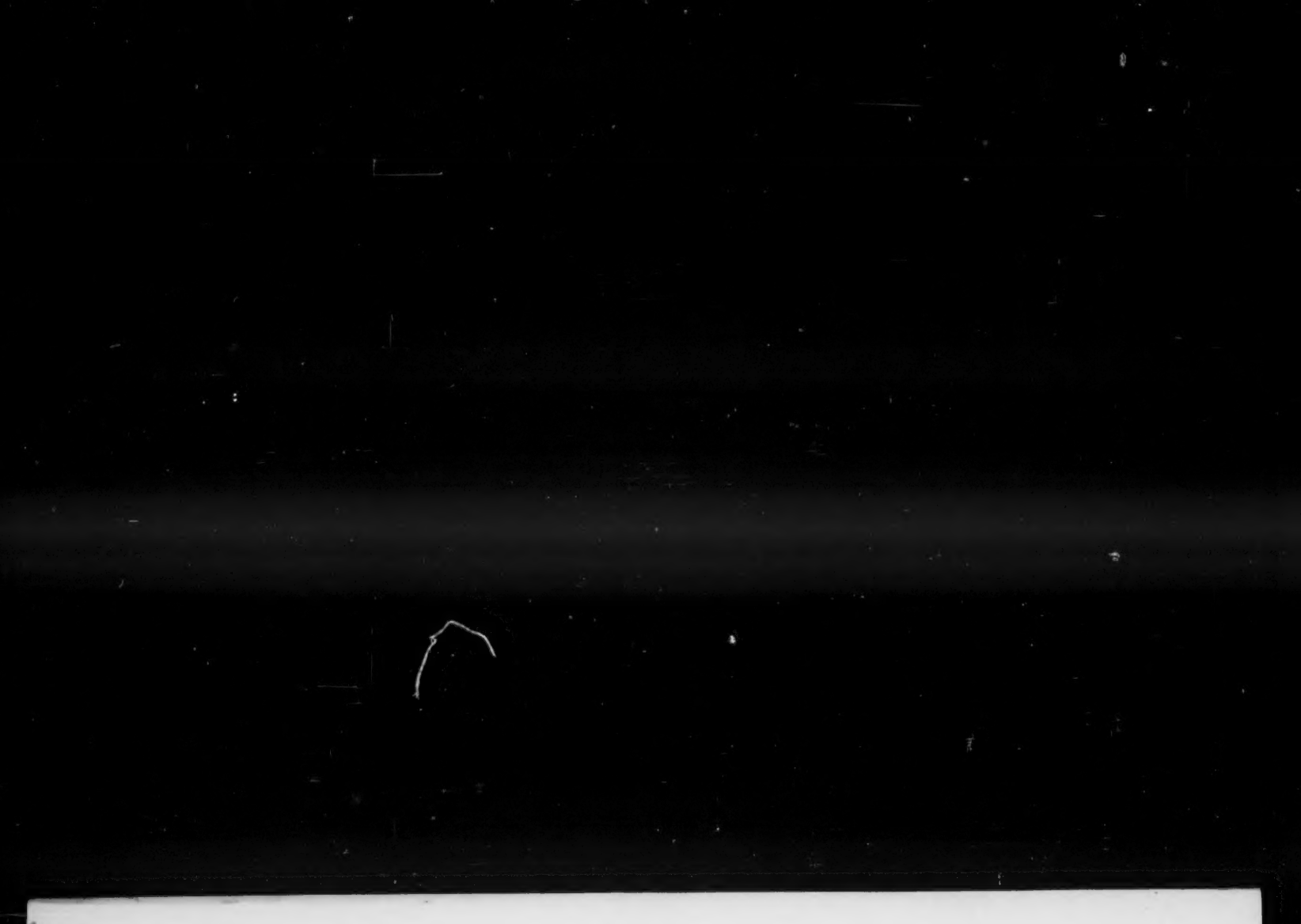
BROOKLYN BOTANIC GARDEN, BROOKLYN, NEW YORK